NORTH CAROLINA

Medical Journal

Vol. 13 No. 4
April, 1952

WILLIAM DEBERNIERE MACNIDER
MEMORIAL ISSUE

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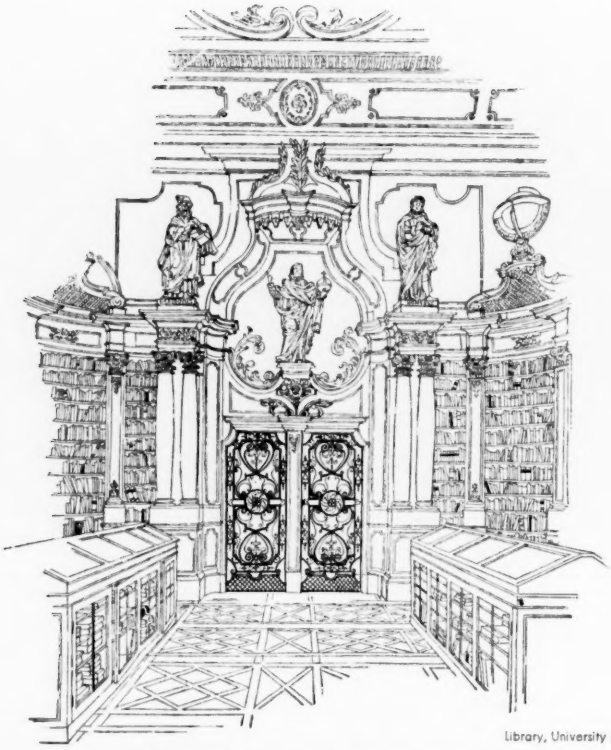

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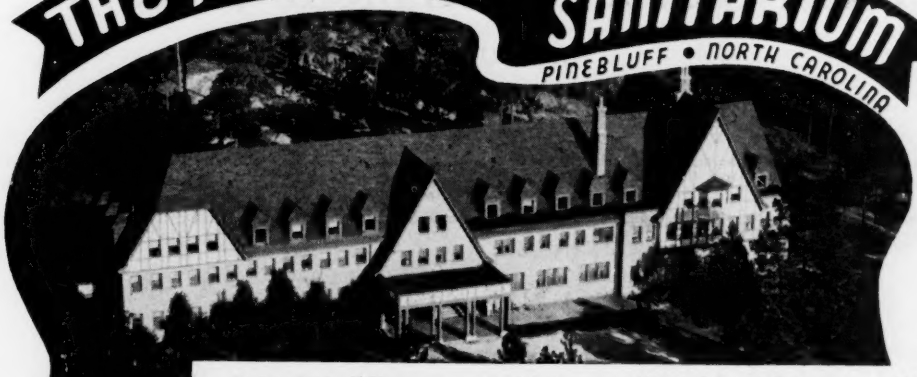

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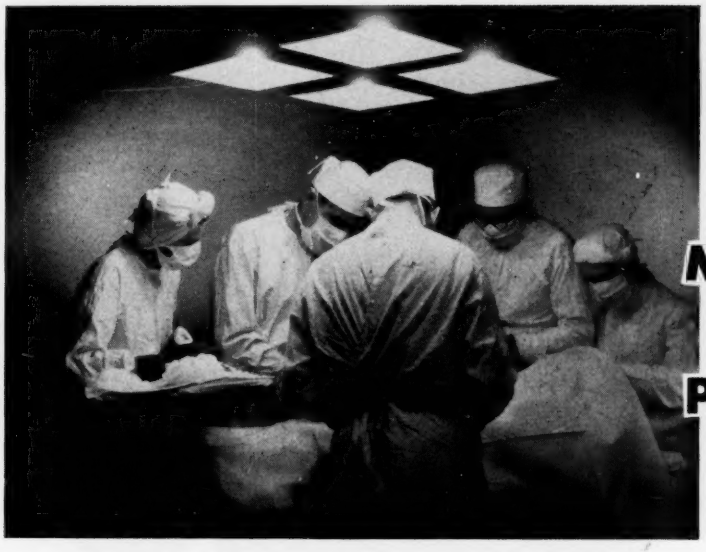

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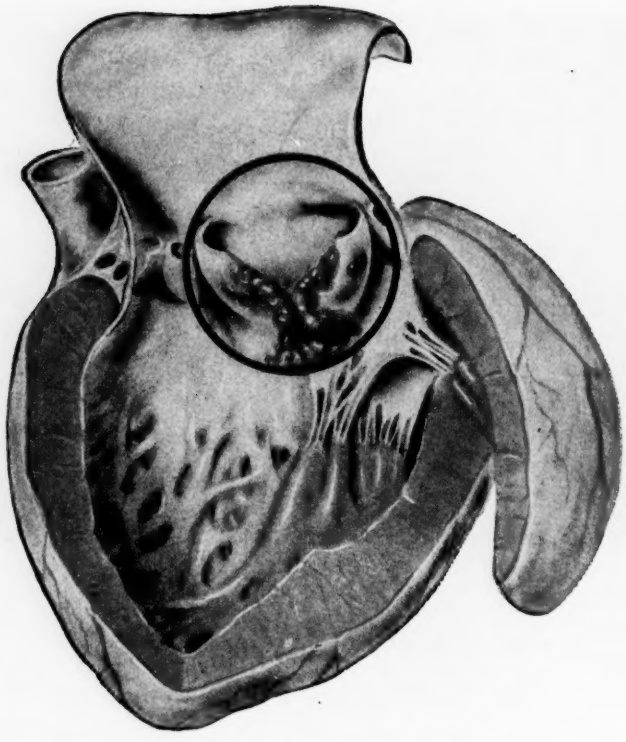

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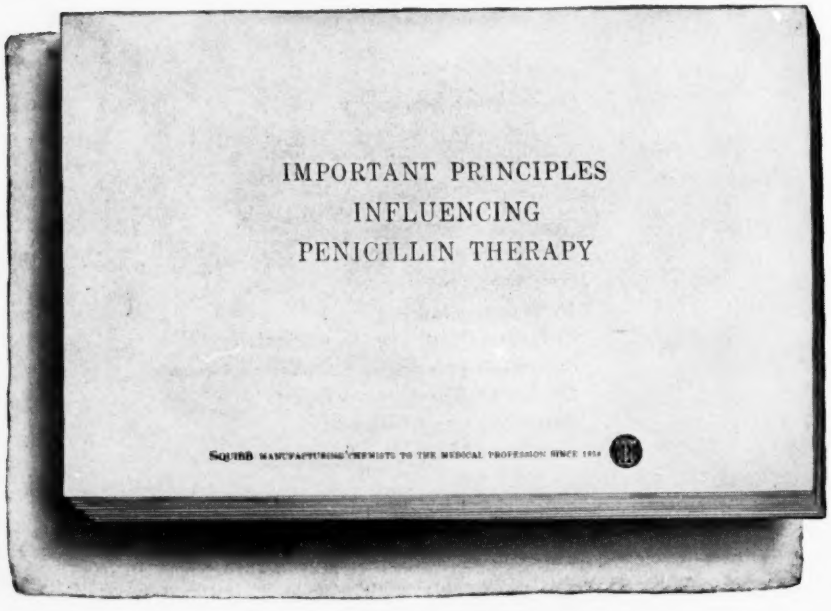

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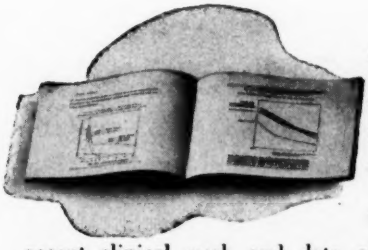

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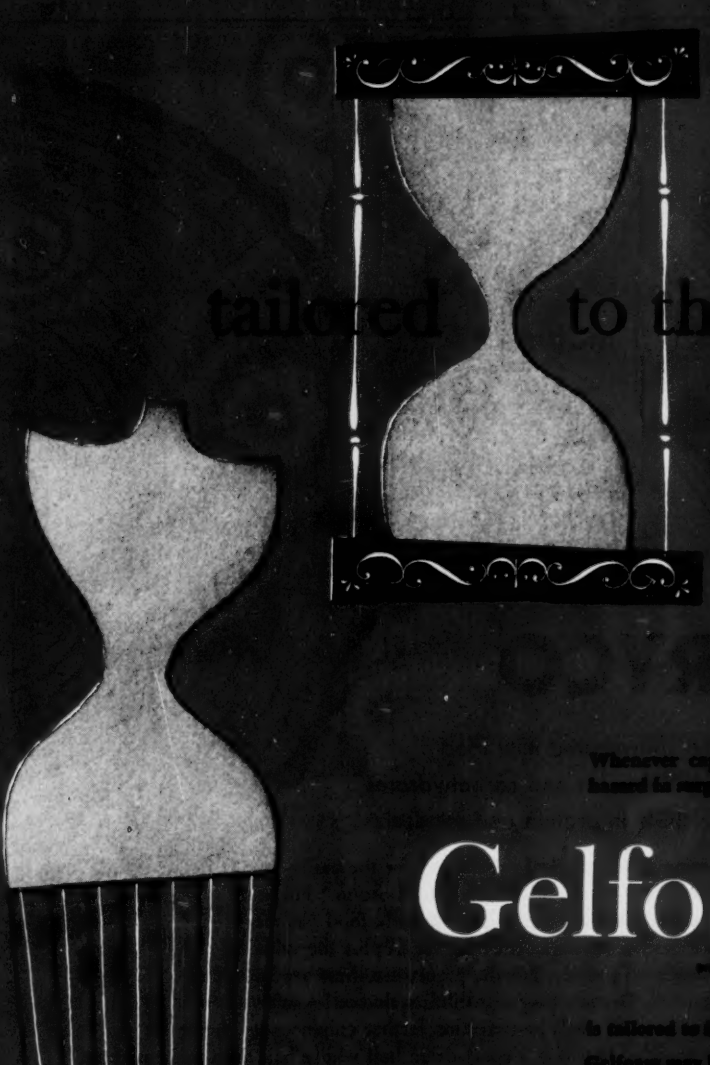

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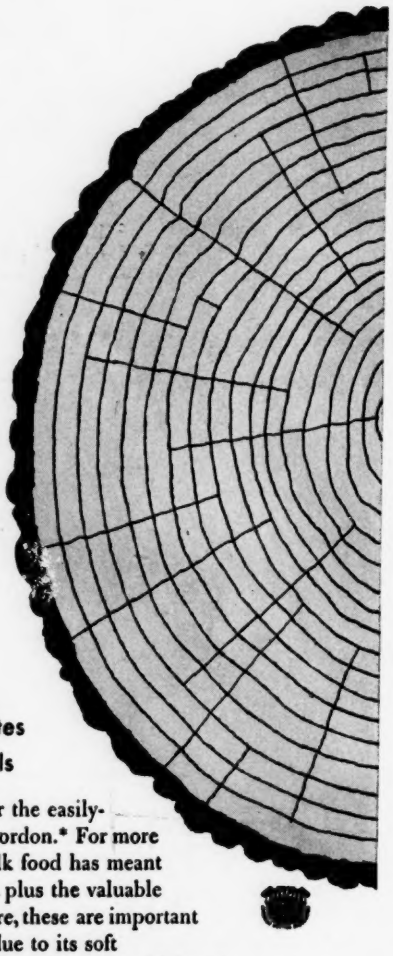

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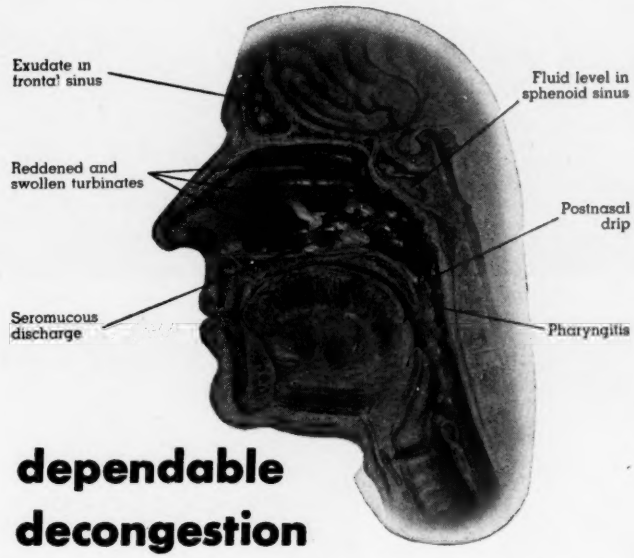

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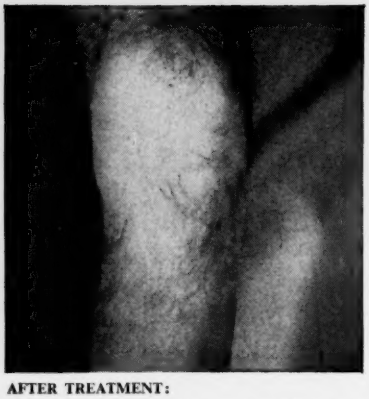

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Ward, L. E., Slocumb, C. H., Polley, H. F., Lowman, E. W., and Hench, P. S.: *Proc. Staff Mtgs., Mayo Clinic* 26: 361, September 26, 1951.

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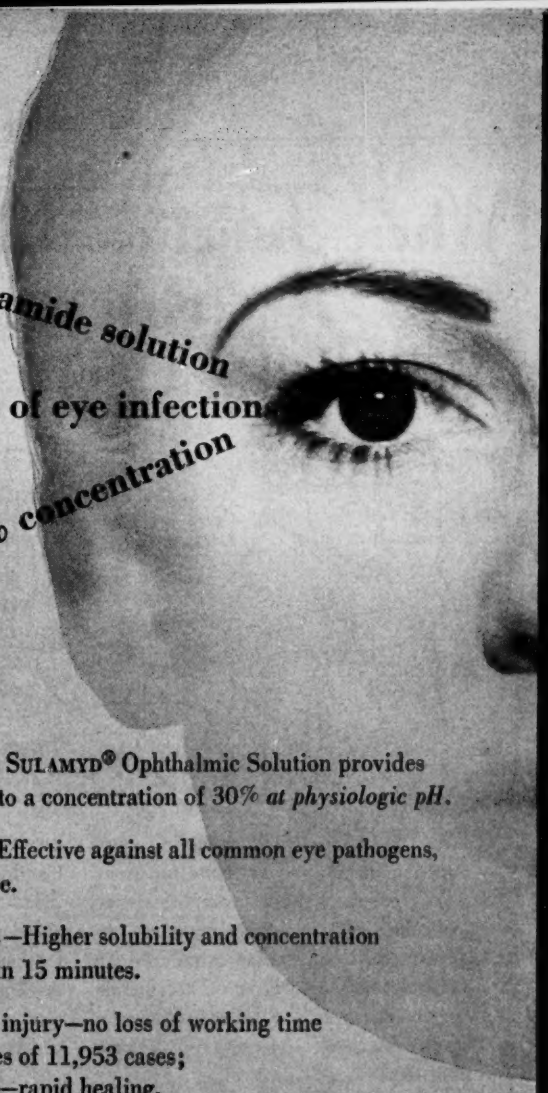

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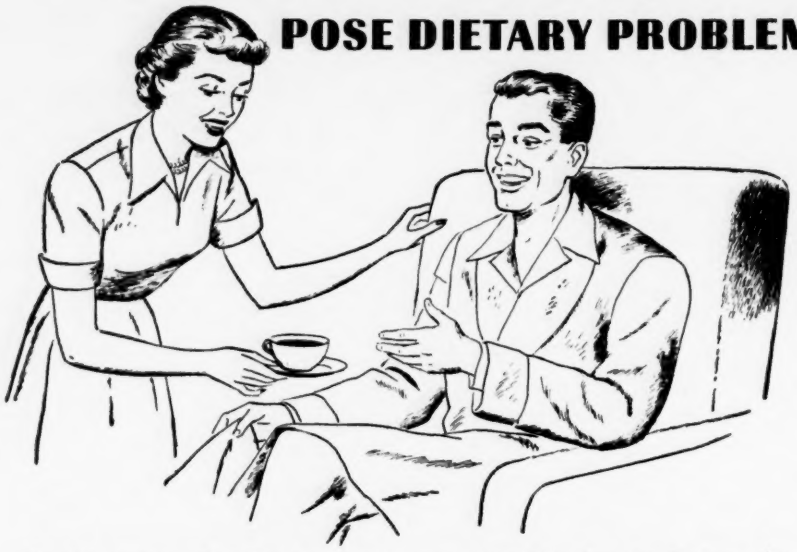

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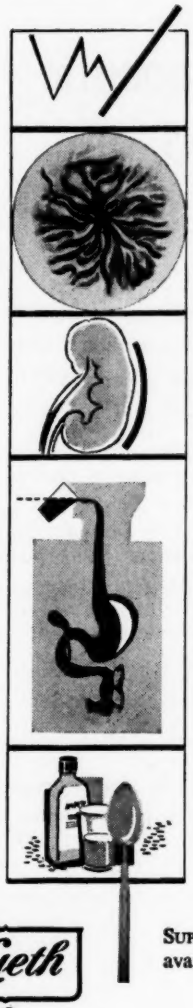

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WILLIAM DEBERNIERE MACNIDER

(1881-1951)

William deBerniere MacNider, son of Virginius St. Clair and Sophia Beatty (Mallett) MacNider, was born in Chapel Hill, North Carolina, June 25, 1881, and died in Chapel Hill, May 31, 1951.

He received his elementary education in the schools of his home town, and his college and professional training at the University of North Carolina. After three years of study in college and the preclinical medical school at Chapel Hill, he transferred to the clinical school of the University that had just been established at Raleigh. He graduated there with the first class of young men to receive degrees of Doctor of Medicine from that institution. His professional education was extended during some subsequent summers through study at the University of Chicago and Western Reserve University.

Throughout most of his life Dr. MacNider was a member of the faculty of the University of North Carolina, being successively assistant in biology (1899-1900), assistant in anatomy (1900-1902), assistant in clinical diagnosis (1902-1905), professor, Kenan professor, and Kenan research professor of pharmacology (1905-1950). He retired as emeritus professor in 1950. From 1937 to 1940 he was dean of the medical school.

Will MacNider's achievements and the force and charm of his personality were recognized by honorary degrees from Davidson College and the Medical College of Virginia, by memberships and official posts in scientific and social groups, and by medals and lectureships. He was a member of the American College of Physicians, the National Academy of Sciences, the American Philosophical Society, and many other scientific organizations. He served as president of the American Society for Pharmacology and Therapeutics, the Medical Society of North Carolina, the Elisha Mitchell Scientific Society, the Society of Experimental Biology and Medicine, the International Anesthesia Research Society, and the Gerontological Society. He was awarded the Gibbs prize of the New York Academy of Medicine, the Research Medal of the Southern Medical Association, and the Kober Medal of the Association of American Physicians.

Dr. MacNider's principal contributions to creative scholarship may be grouped into five categories:

1. The production of various forms of acute and chronic nephritis with cytologic study of the types of injury and of the processes of repair.

2. Disturbances in the acid-base balance in the blood in experimental nephritis during anesthesia and intoxication by salts of heavy metals.

3. The influence of lipoid accumulations within renal cells upon the susceptibility of the cells to the toxicity of anesthetics together with demonstration of the ability of alkalies or glucose to decrease that susceptibility.

4. Studies of the factors of age in the response of animals to normal and abnormal influences.

5. Evidence that when certain epithelial tissues are severely injured by toxic agents, the succeeding repair process forms a typical, yet functionally effective type of cell with an acquired resistance to the chemical agent of injury and other agents of different chemical order.

Being the son of a physician and the grandson of a physician, and living in Chapel Hill at a time when the ferment of modern science was becoming active there, it seems that heredity and environment conspired to make Will MacNider a medical scientist.

As a boy he was interested in nature and as a young undergraduate he acquired an enthusiasm for science. He has said that his enthusiasm was sparked by the study under the microscope of a living kidney tubule of an earthworm. Many another young student has been thrilled by the sight of life in operation on the cellular level, but for young MacNider the thrill was seed in good ground. The spectacular sight of the earthworm's nephridium fired him with a strong inspiration that led on to a passion for searching the secrets of organs, of cells, of tissue fluids, of organisms — to a lifetime of research devoted principally to a study of the function and pathology of the kidney. Although this line of research was never abandoned, his interests broadened to encompass an interest in the fundamental nature of the vital processes of organisms and their philosophical implications.

He was fortunate in early coming under the stimulating influence of some of the inspiring pioneers of science of 50 years ago. H. V. Wilson and Richard Whitehead of Chapel Hill, S. A. Matthews of Chicago, G. N. Stewart and Torald Sollman of Cleveland, and Thayer and Osler of Johns Hopkins University were men with whom he was briefly or long associated and from whom he drew inspiration and stimulus during his early years. His own eagerness and industry in the exciting search for new truth have contributed largely to the intellectual climate of this University throughout the fifty years that he was a part of it.

The list of his publications supplies ample testimony to his untiring activity and his wide interests. His published contributions to science and teaching are on deposit in the medical library. They consist of 20 volumes of bound reprints, a gift from him to the library about a year before his death. His interest in the library was further demonstrated from time to time by gifts of books and journals, by the gift of his extensive collection of reprints from investigators all over the world, and by the bequest in his will of the scientific books remaining in his considerable library.

Although he valued highly the friendship and acclaim of his professional colleagues and contemporaries, his greatest reward was the esteem and affection in which he was held by students that passed under his tutelage. His zest for learning could not make a recluse of him because it was accompanied by an equal zest for companionship that drew students to him in lifelong friendships.

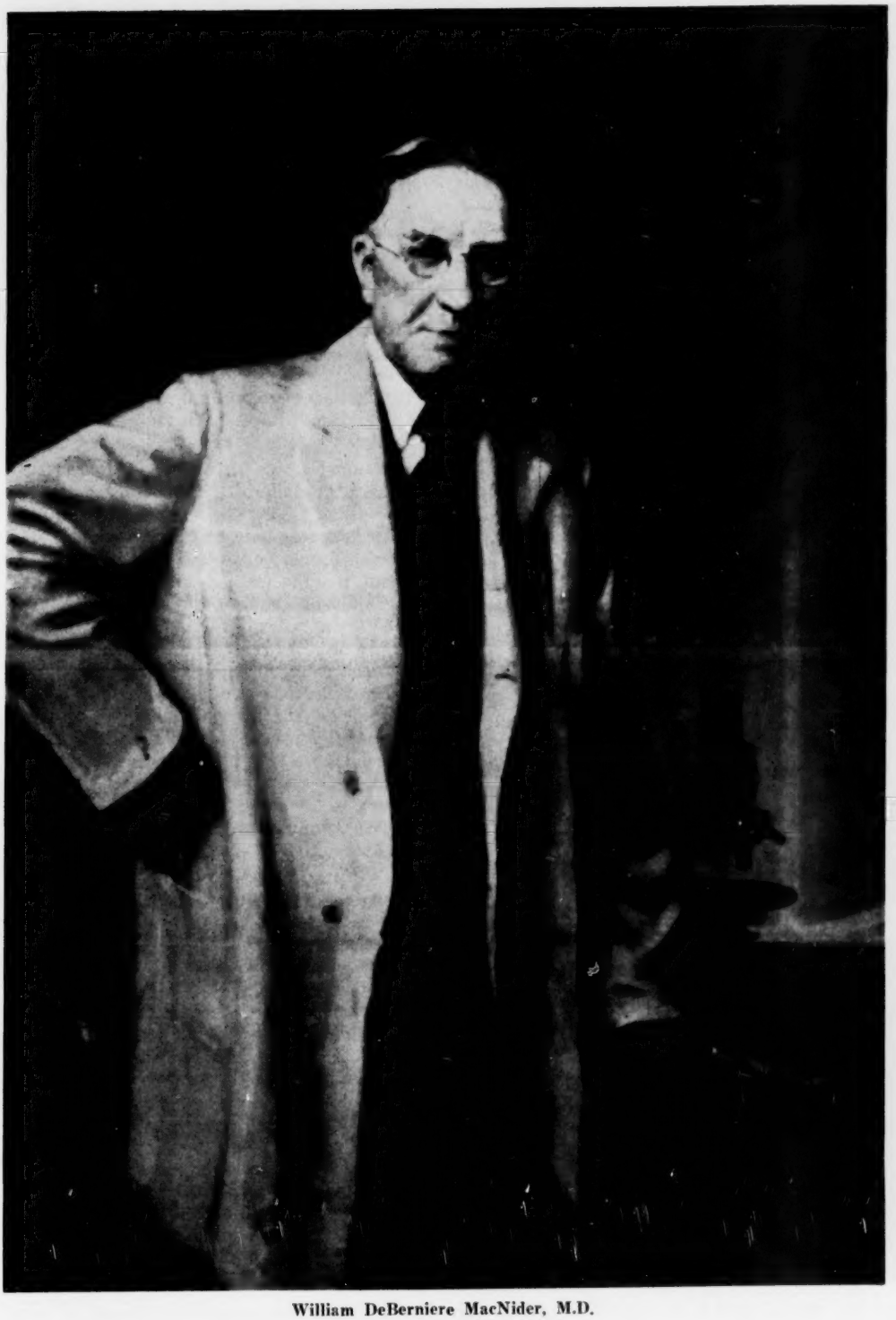
Research and teaching occupied most of his time and energies, and yet enough was left over for a live and active interest in his home, which was the center for much pleasant hospitality. Work in his garden filled the place that sports fill in many men's lives. Gifts of flowers to neighbors and local friends were a frequent occurrence, and many acquaintances throughout the country have had their hearts gladdened and their gardens beautified through gifts of flower seeds from his garden.

Throughout his career he remained profoundly interested in the methods and problems of medical education and in the practical as well as the philosophical implications of scholarships. In the council chamber and committee room he was a forceful exponent of his views, and his opinions were sought and weighed with interest by antagonists as well as by supporters.

Although intensely interested in his own thoughts and his own affairs, Will MacNider had a keen interest in the work, the pleasures, and the sorrows of those with whom he came in contact. He contributed to their pleasures and knew how to be of help in their sorrows. His death leaves a gap in the domain of science and in the lives of hosts of friends.

W. C. GEORGE.

New and better drugs for combatting bacterial diseases may be expected. I envisage the gradual replacement of the drugs which must be administered intravenously or intramuscularly by others of equal or greater potency which may be taken orally. Many of the most stubborn diseases of mankind are those caused by viruses, such as the common cold, poliomyelitis, spinal meningitis, influenza, virus pneumonia, mumps, and measles. Satisfactory drugs for their treatment are lacking. The retarded progress in this field in contrast to that made in the study of bacterial diseases is the result of the absence of suitable laboratory or animal assay methods for determining the effectiveness of any chemical agent upon a particular virus. Bacteria can be grown in the laboratory, but viruses propagate only within living bacteria or living cells. Research in the next decades will solve the vexing problem of finding viruses, and thus open a new chapter in medical therapy.—Roger Adams: Man's Synthetic Future, Science 115:163 (Feb.) 1952.



William DeBerniere MacNider, M.D.
1881 — 1951

NEWER ASPECTS OF LIPID METABOLISM IN ATHEROSCLEROSIS

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For many years interest in the metabolism of blood lipids was focused upon the well established observations that hypercholesterolemia tends to occur in a variety of clinical conditions. It has been associated not only with so-called "senile arteriosclerosis" but also with certain other diseases, especially diabetes mellitus, nephrosis, hypothyroidism, and primary xanthomatosis, all of which are known to predispose to premature atherosclerosis.

Gradually, however, it has become apparent that there is no predictable relationship in man between the height to which the blood concentration of cholesterol rises and the severity of the atheromatous lesions. Furthermore, it is not uncommon for the atherosclerotic process to develop even though the serum cholesterol value is within the so-called "normal" range. Accordingly, many recent investigations have been concerned with other aspects of cholesterol metabolism involving the physico-chemical interrelationships of this sterol with certain other blood constituents. The evidence accumulating from this work suggests that a disturbance in lipid metabolism in general, rather than cholesterol alone, may occur in the patient who shows an atherosclerotic tendency.

The intention of this review is to present some of the newer knowledge of these metabolic changes which appear to be related to the development of experimental and human atherosclerosis. It is recognized here that many influences undoubtedly are involved in the pathogenesis of this disease, but a discussion of other possible etiologic factors and general features of atheromatosis will not be attempted.

Production of Experimental Atherosclerosis

Since the initial observation by Anitschkow[1] that cholesterol feeding led to the development of atheromatous lesions in rabbits, several additional species have been found to exhibit varying degrees of this susceptibility. At the present time, the rabbit, chicken, and dog are used most frequently for the production of an atherosclerotic state which resembles the biochemical and pathologic characteristics of human degenerative vascular disease. Inasmuch as some of the data to be discussed have been derived from studies of animals, it seems desirable to comment briefly on some of the procedures used to produce this experimental condition.

Rabbits are particularly sensitive to exogenous cholesterol. The ingestion of this sterol for several weeks usually produces a marked elevation of serum cholesterol, which is disproportionate to the increase in other serum lipid fractions, and a subsequent development of localized atheromatous lesions, especially in the arch and thoracic portion of the aorta[2]. The opinion has been expressed, however, that these studies may not be pertinent to man, since the herbivorous rabbit does not normally ingest cholesterol and probably has a very low metabolic capacity for its disposal. Nevertheless, investigations in this species have yielded valuable data relative to the general nature of atherosclerosis.

Katz and co-workers[3] have demonstrated that the chicken, an omnivorous animal, upon ingesting a cholesterol-fortified diet, readily develops atheromas which resemble human lesions much more closely in character and distribution than do those in rabbits. In virtually every case, the level of serum cholesterol and incidence of atherosclerosis were related directly to the amount and duration of cholesterol intake. On the other hand, induced lesions occurred in some chicks with minimal hypercholesterolemia[4], while many aging chickens, even on a low-fat, cholesterol-free diet, developed spontaneous lesions[5] which were similar to those induced by cholesterol. These workers have shown also that estrogen administered to cholesterol-fed chickens inhibited coronary, but not aortic atheromatosis[6], whereas estrogen treatment alone resulted in a delayed appearance of hypercholesterolemia and ultimate development of atheromatous lesions[7].

Feeding cholesterol to normal dogs does not usually elevate blood cholesterol or produce atherosclerosis. Steiner and Kendall[8] discovered, however, that thiouracil suppression of thyroid function along with cholesterol feeding for several months did result in the development of high serum cholesterol and atheromatous lesions. These le-

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sions have the same distribution and sites of predilection, as well as most of the morphologic features, as those which occur in man. Recent data suggest that incipient spontaneous changes in the intima of aging dogs may be analogous to lesions induced by the cholesterol-thiouracil regimen[9]. Conversely, thyroid hormone decisively retards hypercholesterolemia and atherosclerosis in cholesterol-fed and estrogen-treated animals[10].

Present Status of Hypercholesterolemia

Relation to diet

It is obvious that the hypercholesterolemia of experimental atherosclerosis is induced by excessive cholesterol intake, although, as previously mentioned, simultaneous thyroid depression is necessary in dogs. In man, it is less certain what role the consumption of a cholesterol-rich diet plays in raising the blood concentration. A clear interpretation of this point is complicated by the fact that cholesterol is synthesized in the body. Furthermore, the "normal" range of serum cholesterol in human subjects is quite wide.

One view held[11] is that dietary cholesterol exerts an important influence on blood concentration and ultimately on the development of atherosclerosis. However, many other workers share a more recent opinion which opposes this hypothesis. For example, Keys[12] studied the influence of diet on blood cholesterol levels in 482 "clinically normal" men ranging in age from 18 to 55 years. His results show that if the habitual daily intake stays within a range of 250 to 800 mg. of cholesterol, there is no significant relationship to serum concentration. In one group of 41 men who had a mean initial value of 250 mg. per 100 ml., 50 per cent reduction in dietary cholesterol for several months failed to change the control level. However, on the Kempner rice diet[13], which is cholesterol-free and nearly fat-free as well, the mean value fell from 323 mg. per 100 ml. to 151 mg. per 100 ml. within three weeks. Keys concludes that "the blood cholesterol level is independent of the intake over a wide range, but that at zero intake it falls at a rate related to the previous serum level."[12]

Furthermore, Gertler, Garn, and White[14] found no correlation between the amount of ingested cholesterol and the serum concentration in a study of 139 healthy men and 90 males who had experienced myocardial infarction. The habitual cholesterol intake of the control group was significantly higher than that of the coronary patients. Also, Steiner and Turner[15] were unable to detect significant changes in serum cholesterol within 24 hours after a single large meal rich in cholesterol and fat. Their results were similar when high and low cholesterol-fat diets were extended over a period of several weeks.

Thus it is seen that the average cholesterol intake, and probably the low-cholesterol diets also, have relatively little control over the blood level, but if a stringent cholesterol-free diet be consumed regularly, an unequivocal fall in cholesterol concentration follows. In order to accomplish this result, it should be emphasized that a low-fat diet may be more important than the deprivation of cholesterol. To this end it has been shown that the serum cholesterol reduced by dietary restriction returns fairly rapidly to the original level upon the addition of vegetable (non-cholesterol) fat to the diet[12,16]. Perhaps this can be explained on the basis that endogenous cholesterol excreted into the intestine is effectively reabsorbed only after ester formation with fatty acids[17]. It becomes clear, then, that if dietary control is to be attempted as a measure to reduce the cholesterol content of blood, a very drastic limitation of cholesterol and fat will be required.

Relation to incidence of atherosclerosis

In most instances of experimental work, elevation of serum cholesterol appears to be a prerequisite for the development of atherosclerotic lesions. The occurrence of spontaneous atheromas in chickens, and possibly in dogs, is an exception to this generalization. Much of the clinical literature indicates a definite trend toward atherosclerosis in patients who manifest hypercholesterolemia, although this relationship is not uniformly present. Several workers[14,15,18] have observed an increase in mean blood level in patients with atherosclerotic disease. On the other hand, many patients so affected have serum cholesterol values within the normal range. Thus in a study of 200 consecutive patients with acute coronary thrombosis, hypercholesterolemia was present in 68 per cent of 75 patients under 60 years of age, but in 52 per cent of 125 patients over the age of 60 years, a normal cholesterol level was found[19]. Gubner and Ungerleider[20] reported the incidence of atherosclerosis to

be about the same in patients with mild hypercholesterolemia (average 300 mg. per 100 ml.) and in subjects with normal serum cholesterol (average 211 mg. per 100 ml.). Among persons showing low levels (average 160 mg. per 100 ml.) there was a significantly lower incidence.

Fluctuations in serum cholesterol may bear a more significant relationship than sustained high concentrations *per se* to the incidence of atherosclerosis. Thus Morrison and others[21] reported a variation of less than 10 per cent in individual cholesterol values of normal subjects over a period of one year, whereas there were wide deviations in the levels of 50 patients with recent coronary thrombosis. Steiner[15] also observed a similar variation in individual atherosclerotic patients. These data are compatible with the view[22] that human atherosclerosis may advance in stages as the result of repeated insults, rather than as a gradually progressive deterioration.

The fact that a higher incidence of atherosclerosis occurs in individuals who exhibit some derangement in cholesterol metabolism is indisputable. Yet simple elevation of serum cholesterol does not appear to be the most significant factor, and, on this basis, the evidence is not sufficient to justify assumption of a causal relationship. It has been suggested[20] that perhaps hypercholesterolemia is only a manifestation of an existing metabolic abnormality, the most outstanding stigma of which is the causation of atherosclerosis.

Physico-Chemical State of Blood Lipids

From the foregoing discussion it seems evident that the serum cholesterol concentration alone has proven to be an unreliable criterion of the incidence or severity of atherosclerosis and, indeed, has failed to provide the etiologic clue to this disease. It seems to be equally true that mere concentration of any serum lipid is meaningless. As an alternative approach to the solution of this problem, research efforts in general have shifted to a rather intensive investigation of the physico-chemical state of blood lipids, with particular reference to their particle size and the nature of their interrelated functions. Hueper[23] has emphasized the possible etiologic significance of large colloidal particles in the blood stream. He demonstrated, by intravenous injection into animals, that polymers of high molecular weight substances ("macromolecules"), such as polyvinyl alcohol, methyl cellulose, pectin and acacia, gradually accumulated in the arterial intima and gave rise to lesions morphologically similar to those of atheromatosis.

Subsequent investigations seem to confirm this general concept to the extent that coarse particulate distribution of serum lipids apparently predisposes to their intimal deposition and, thereby, probably plays an important role in the inception of atheroma formation. Some of the current ideas relating to this concept of the initiation of atherosclerosis will be presented now.

Cholesterol-phospholipid ratio

Many recent studies have called attention to the possibility that the relative concentration of phospholipids assumes a dominant role in stabilizing the colloidal serum lipids. This relationship is usually represented by the *ratio* between the concentrations of cholesterol and phospholipids. Normally this value is approximately 1 when cholesterol is expressed as milligrams per 100 ml. and phospholipids are expressed as lecithin milligrams per 100 ml. (The phospholipid value may be obtained by multiplying serum lipid phosphorus by a factor of 25.)

Davidson, Abell, and Kendall[24] believed a shift of the cholesterol-phospholipid ratio from 1 in young dogs on a normal diet to 5 in animals on a cholesterol-thiouracil regimen was closely associated with the development of atherosclerosis in the latter. They observed a marked hypercholesterolemia and a less marked rise in phospholipids which resulted in an elevation of the ratio.

It has been reported[25] that the intravenous injection of certain synthetic detergents (Tween 80 and Triton A-20) in cholesterol-fed rabbits produced a sustained elevation of serum phospholipids which kept pace with the anticipated hypercholesterolemia. In these animals the normal ratio was preserved, and there was a significant decrease in the incidence and severity of atherosclerosis as compared with the uninjected cholesterol-fed controls. Payne and Duff[26] recently expressed doubt that this inhibitory effect is due merely to elevation of phospholipids, but believed that Tween 80, owing to its prolonged stay in the blood stream, might prevent the development of experimental

atherosclerosis through a mechanism independent of lipid changes.

It has been shown by Duff and McMillan[27] that alloxan diabetes completely inhibits the usual cholesterol-induced atherosclerosis in rabbits. This experimental observation is at variance, of course, with the evidence in man that diabetes mellitus favors the early development of atheromatosis. However, this unanticipated response has been confirmed[28] and seems well established. In these animals hypercholesterolemia developed in the characteristic manner, but it was accompanied by a commensurate elevation of phospholipids. The mechanism by which atherosclerosis was inhibited appeared to be related to the maintenance of the cholesterol-phospholipid ratio in spite of the high rise in serum cholesterol[29]. Insulin regulation of the diabetic state abolished the inhibitory effect and atherosclerosis developed as in the nondiabetic cholesterol-fed rabbits[30].

According to the data of Pomeranze and Kunkel[31], in human diabetes there is a better correlation of the incidence of atheromatous lesions with an increased cholesterol-phospholipid ratio than with either hyperlipemia or elevated serum cholesterol. Hence, it appears that, regardless of the peculiar difference in influence of diabetes and its control in experimental and human atherosclerosis, an important determinant in the development of atheromatous lesions is the extent to which the normal cholesterol-phospholipid ratio becomes altered.

On the basis of lipid-pattern determinations in several conditions associated with hyperlipemia, it has been shown[18,32] that the cholesterol-phospholipid ratio is elevated above normal in those cases that commonly display atherosclerosis (cholesterol-fed rabbit, hypothyroidism, essential xanthomatosis, nephrosis), whereas in biliary obstruction, a condition in which the incidence of atherosclerosis is not increased although characteristically a marked hypercholesterolemia is present, there is a very significant reduction in this ratio. Gertler and coworkers[33] have found an increase in this ratio also in patients with coronary artery disease. They advanced the hypothesis that the levels of serum cholesterol and phospholipids as independent values are less important in atherosclerosis than is the ratio of cholesterol to phospholipid.

During prolonged estrogen administration to chicks, atheromatosis was retarded initially, presumably by a predominant rise in phospholipids. However, atherosclerosis developed eventually in the presence of a persistant hypercholesterolemia[10b]. When cholesterol feeding and estrogen injection are combined, a parallel increase in cholesterol and phospholipids occurs and the ratio remains normal[6]. Under the latter conditions coronary atherogenesis is inhibited. Eilert[34] observed a decided drop in the cholesterol-phospholipid ratio of female patients during estrogen therapy. She feels that the lower incidence of coronary artery disease in women before onset of the menopause might be attributable to higher estrogen activity.

Thus a great deal of evidence is available to favor the concept that phospholipids, being hydrophilic, are capable of holding in suspension the hydrophobic lipids, and hence, of maintaining these colloidal particles in a finely dispersed state. The proponents of this theory feel that the simple elevation of cholesterol or total lipids is not detrimental so long as there is a concomitant rise in phospholipids, with no increase in the cholesterol-phospholipid ratio.

Chylomicronemia

Normally in the fasting state, or after a fat-free meal, fat particles are relatively few in number and small in size in human serum when examined by dark-field microscopy or measured by the technique of nepholometry. However, following the absorption of fats from the intestinal tract, there appear in the serum relatively large and numerous particles which are called "chylomicrons"[35]. These particles are not a reflection of the serum lipid concentration, but represent an alteration in the physical state of absorbed fat.

The intensity and duration of this postprandial hyperchylomicronemia vary not only with the amount of ingested fat, but with certain other seemingly significant factors. Becker and others[36] reported that young normal individuals showed only a moderate chylomicronemia which usually disappeared within three to five hours following the ingestion of a standard fat meal. They observed an increase in intensity and duration with advancing age, and in patients over 50 years of age the chylomicron count was very high and gradually disappeared only after 24 hours. In this older age

group it was predicted that under the influence of regular eating habits these subjects would carry a persistent hyperchylomicronemia from meal to meal.

Moreton, who recently revived interest in chylomicrons by postulating their possible role in the pathogenesis of atherosclerosis, has observed a sustained hyperchylomicronemia in cholesterol-fed animals and in patients afflicted with diseases associated with the atherosclerotic process[22]. Zinn and Griffith[37] have confirmed this observation in victims of myocardial infarction and in diabetic persons of random selection. In the fasting sera of these patients, chylomicrons constituted over half the number of fat particles detectable by dark-field illumination. Moreton believes that the increase in chylomicrons accompanying alimentary hyperlipemia is essentially indistinguishable from the chylomicronemia of experimental and human atherosclerosis. Accordingly, he has advanced the theory that "the gradual development of atherosclerosis in otherwise normal human beings may be the direct result of recurrent alimentary chylomicronemia due to repeated ingestion of many fatty meals over a lifetime." It is postulated further that these coarse cholesterol-containing lipid particles become entrapped in the arterial intima, from which location neutral fats and fatty acids are more easily removed than cholesterol, and, consequently, the latter substance accumulates at the sites of potential atheromas.

More recently Moreton has described a fat tolerance test which constitutes a method for determining the chylomicronemic response to ingested fat[38]. This response shows a wide variation among normal persons, but tends to remain in a specific individual relatively constant over a period of months. By this test serum cholesterol and chylomicronemia do not correlate well, although some degree of hypercholesterolemia is usually present in patients who show an increase in chylomicrons. Many atherosclerotic patients show poor tolerance to ingested fats, and elimination of these dietary items usually results in a rapid and marked reduction in the "natural" chylomicron curve even when the habitual level is quite high.

Lipoproteins

A significant recent contribution is the work of Gofman and colleagues[39], who have used the ultracentrifuge and a special flotation technique to demonstrate the presence in serum of certain newly defined lipid-bearing giant molecules which show a remarkable correlation with the incidence of experimental and human atherosclerosis. According to this work, "essentially none of the serum cholesterol circulates as individual molecules of either free or esterified cholesterol but instead is present in the form of very large molecules containing cholesterol and other lipids in association with variable amounts of proteins." These lipoproteins are segregated on the basis of their relative densities and are classified as Sf molecules (Svedberg units of flotation) according to their characteristic flotation rates. At the present time, characterization has been accomplished for discrete components occurring at frequent intervals from Sf 2 through Sf 17, and for a homogeneous series between Sf 17 and Sf 40,000.

In human sera, classes of giant molecules representing the whole gamut of the lipoprotein spectrum have been identified[39c]. Age and dietary habits appear to modify a particular individual's capacity to metabolize lipids, which is thought to determine his lipoprotein pattern. Molecules as high as Sf 8 are considered "normal" in young human subjects under the age of 25 years. Beyond this age there was a variable increase in abnormal molecules of higher Sf values in persons representing the general population. The ingestion of fatty meals was followed in most subjects by serum elevation of various abnormal giant molecules. Whether this becomes a transient or sustained phenomenon in a given person appears to depend upon the integrity of his lipid metabolic mechanism.

By far the most significant group of lipoproteins in man is the Sf 12-20 class, the serum concentration of which correlated strikingly with the incidence of atherosclerosis in a large number of patients with coronary artery disease, diabetes mellitus, nephrosis, and hypothyroidism. No other class of molecules exhibited this relationship. Cholesterol levels were related to atherosclerosis in these patients only to the extent that Sf 12-20 molecules contained the major fraction of the serum concentration.

The opinion is held by Gofman that the serum level of Sf 12-20 molecules in apparently normal individuals is of diagnostic

value in predicting their likelihood of developing a clinical manifestation of atherosclerosis. Furthermore, it is believed that this criterion has prognostic significance in patients who have already displayed evidence of this disease. In patients with coronary disease, recurrent myocardial infarction occurred earlier if high levels of these lipoproteins were maintained, whereas there were fewer recurrent episodes in those individuals whose serum levels were reduced by prophylactic measures.

In rabbits, normal lipoproteins are represented by classes up through Sf 8-10. During cholesterol feeding experiments, these molecules increased to a certain concentration, and then lipoproteins of higher Sf values began to appear[39a]. These atypical classes are thought to indicate an "overflow" above the normal capacity of the rabbit to metabolize excessive cholesterol. Throughout these experiments there was an extremely high correlation between the concentration of Sf 10-30 molecules and the severity of atherosclerosis. In fact, an occasional animal failed to develop an appreciable level of this type molecule and, at autopsy, exhibited only minimal atherosclerotic lesions. As in the human, cholesterol levels were related to atherosclerosis only insofar as the incriminated molecules accounted for a high proportion of the total serum cholesterol.

These workers have demonstrated two procedures, dietary restriction and heparin administration, which appear to shift an atypical lipoprotein pattern in the direction of normalcy. On a low-fat, low-cholesterol diet for 16 weeks, normal subjects showed a consistent trend toward a lower level of abnormal molecules, and within four weeks after termination of the diet, this level began to rise toward the initial concentration. Reduction in total fat intake during a period of one year resulted in an appreciably lower blood level of Sf 12-20 substances in one group of patients with myocardial infarction. In this group there was significant protection against recurrent infarction.

Heparin administration[39c, 40] to cholesterol-fed rabbits was associated with diminution in concentration of Sf 10-30 molecules and a conspicuous inhibition of atherosclerosis. In man, heparin injection was followed by depletion of molecules above Sf 20, a marked decrease in the Sf 10-20 class, and a rise in concentration of molecules below Sf 10. Simultaneously, heparin afforded symptomatic relief in patients with severe angina pectoris. This relief was of sufficient duration to suggest a third mechanism independent of the known anticoagulant and vasodilator effect of this drug. The true implication of this observation and the involved mechanism await clarification.

There has emerged from these studies the concept that abnormal lipoproteins are products of aberrant pathways of lipid metabolism, and that a sustained serum elevation of molecules with higher Sf values than those of the normal range probably is indicative of an error in the normal metabolic pattern. Furthermore, there is thought to be a parallel between the sequential appearance (and concentration) of higher Sf molecules and the progressive severity of the impaired metabolism. Inasmuch as certain types of abnormal molecules can be correlated significantly with the incidence of both experimental and human atherosclerosis, it has been postulated by Gofman that these particular lipoproteins play a fundamental role in the genesis of atheromatosis.

In his critical analysis of Gofman's data, Keys[41] claims that Sf 12-20 blood levels correlate no better than serum cholesterol values with the degree of atherosclerosis. Indeed Keys doubts that giant molecules have any special significance beyond that for simple cholesterol measurements in the prediction or detection of atherosclerotic activity. With this opinion Keys occupies a minority position, inasmuch as these ultracentrifugal studies are considered generally to represent a signal advance in the over-all effort to bring atherosclerosis within the sphere of human comprehension.

Summary

It seems apparent now that there is no consistent correlation between the serum cholesterol concentration and the incidence of atherosclerosis. Consequently, the fundamental predisposing factor in this disease is no longer considered to be a matter of impaired cholesterol metabolism alone. On the other hand, it is becoming more evident that a disturbance in lipid metabolism in general is involved in this pathogenic process.

Recent studies indicate that the various lipid and lipoprotein components of serum are transported normally in a physico-chemical state of equilibrium which maintains lipid material in a finely dispersed stable

"emulsion." It is believed that any deviation from normal lipid metabolic pathways of sufficient magnitude to unbalance this stabilizing mechanism results in the aggregation of abnormal colloidal lipid-bearing particles which are susceptible to deposition in the arterial intima.

At least three indications of such a metabolic defect have been related recently to the etiology of atherosclerosis. These are (1) elevation of the serum cholesterol-phospholipid ratio, (2) sustained chylomicronemia, and (3) certain abnormal serum lipoproteins in the form of giant molecules. The etiology of the faulty mechanism which produces these changes is obscure at the present time. However, involvement of some hormonal deficiency or imbalance is suggested by the close association of degenerative vascular disease with certain states of altered endocrine function, and by increasing evidence that the metabolism of lipids is influenced by the action of various hormones.

Thus it is possible that atherosclerosis may be shown ultimately to be a metabolic disease initiated by abnormalities in lipid metabolism which result from certain endocrine dysfunctions. Under these conditions, an excessive fat intake imposed on a pre-existing metabolic deficiency in a given individual might become an important determinant of his susceptibility to atherosclerosis. In this connection, it has been demonstrated that certain atherosclerotic patients benefit from dietary restriction of fats. This metabolic concept does not exclude contributions of other factors to the pathogenesis of this disease.

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LABORATORY DIAGNOSIS OF LYMPHOGRANULOMA VENEREUM

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During the past decade evidence has accumulated which indicates that the incidence of infections by the virus of lymphogranuloma venereum is considerably greater than previously reported. Procedures for isolation of the virus and serologic diagnosis have been improved, and several studies indicate a high specificity for such tests. A review of the techniques now available for general use, and a new emphasis on the widespread occurrence of this infection appear desirable.

Lymphogranuloma venereum, also called climatic bubo, lymphogranuloma inguinale, and so forth, is an infectious disease caused by a virus of the psittacosis-lymphogranuloma venereum group. Most of these agents are primarily parasitic in birds, being occasionally transmitted to man with a resultant pneumonitis. Lymphogranuloma venereum however, occurs in nature exclusively in man, where it is transmitted usually by sexual contact. In typical cases infection by this virus is followed by the appearance of rather small solitary primary lesions on the shaft, corona, frenum, or prepuce in the male, and on the labium minus, vaginal wall, or cervix in the female. These lesions are small and may remain unnoticed, or may not occur.

The secondary manifestations are usually limited to the lymphatic network associated with the genitalia. The most common such lesions in the male are slowly developing inguinal buboes. In a considerable number of cases these lymph nodes suppurate. In the female, the infection may spread from vaginal and cervical lesions to the pelvic lymph nodes, following which such manifestations as rectal strictures and fistulas may occur. In both sexes, acute systemic, ocular, respiratory, and central nervous system infections have been reported.

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Incidence

The evidence that infections by this virus may be asymptomatic or occur with a minimum number of symptoms should be emphasized, however. Beeson and Miller[1] have presented serologic evidence of past or active infections in 40 to 45 per cent of Negro patients above the age of 15 years. Of 497 patients of all ages in their group, 33 per cent showed evidence of contact with this virus. A considerably lower but significant incidence was found in white patients. In each group, there was a sharp increase in incidence above the ages of 15 to 20 years. Approximately 60 per cent of the sera from colored patients with a positive serologic test for syphilis were positive in the complement fixation test for lymphogranuloma venereum.

The frequent concurrence of lymphogranuloma venereum and other venereal diseases has been shown by other investigators also. Shaffer and Rake[2] reported that complement fixing antibodies for lymphogranuloma venereum were seldom found in sera from persons with congenital syphilis, but 57 of 97 patients with acquired syphilis, 3 of 6 with chancroid, and 9 of 19 persons with gonorrhea showed serologic evidence of infection by lymphogranuloma venereum. Wall, Heyman, and Beeson[3] reported that over one half of a group of 45 patients with proved chancroid likewise gave evidence of past infection by lymphogranuloma venereum, as shown by results of the complement fixation test.

Wright and his associates[4] reported the incidence of positive skin tests and serologic tests as about 28 per cent in 1192 Negro patients and 18 per cent in 192 white patients admitted to a municipal hospital. Forty-two per cent of 451 Negro factory workers in Virginia recently tested by Luger[5] were found to give positive complement fixation tests, with 14 per cent of these persons having titers of 1:40 or greater. Comparable results have been reported by Wetherbee and associates[6].

Specific Laboratory Diagnostic Techniques

Three specific methods of value in the diagnosis of lymphogranuloma venereum have

been developed. These are direct isolation of the virus, the complement fixation test, and the skin test. The latter method, though not a laboratory procedure, is reported here because of its relation to the serologic status of the patient.

Virus isolation

Pus and biopsy material containing the virus are infectious for mice following intracerebral inoculation, and for the chick embryo following injection into the yolk sac. Rake[7] suggests the addition of 1,000 units of penicillin and 3,000 units of streptomycin per milliliter of the specimen when bacterial contamination is encountered. As the virus may fail to produce visible signs of infection in the first passage in either mice or eggs, subsequent blind passages should be made. If the virus is not established by the second or third passage, further attempts are generally useless. Wall[8] has found that mouse inoculation is preferable, as mice are more resistant to bacterial contamination in the inoculum than are chick embryos, and generally show earlier signs of infection.

This method is somewhat expensive and time-consuming, of course, and is not practicable except in properly equipped laboratories. The virus is highly infectious for laboratory workers, and should be handled only with proper facilities and by experienced personnel.

Skin test

Frei[9] discovered in 1925 that the injection of heated bubo pus from a patient with this disease into other infected individuals provoked a sensitivity reaction similar to the tuberculin reaction. Infected mouse brain antigens have likewise been suggested but have proved unsatisfactory owing to nonspecific reactions as shown by control injections of normal mouse brain antigens. The material in general use today for the skin test consists of killed virus prepared from infected chick embryo yolk sacs. Control antigen prepared from normal yolk sacs should always be injected simultaneously with the virus antigen.

The test consists of the intradermal injection of 0.1 ml. each of virus antigen and control antigen in the flexor surface of the forearm. Readings are made after 48 hours. The surrounding erythema should be ignored and only the central papule measured. In the absence of sensitivity to the normal antigen, a papule of 6 mm. or greater in diameter is significant[7]

In general it appears that the skin test becomes positive after complement fixing antibodies are detectable, but remains positive for many years or for life. The specificity of this test, especially in individuals with syphilis, has been challenged by Knott and associates[10]. Other evidence[4, 11], however, indicates that with the use of control antigens the specificity is high, although the sensitivity may be less than that of the complement fixation test.

Because of the common or group antigens in the psittacosis-lymphogranuloma venereum group of viruses, persons infected with psittacosis may show a positive skin test. Bedson and colleagues[12] have prepared acid-extracted antigens of both lymphogranuloma venereum and psittacosis viruses which are highly specific. They found no cross reactions in a small series of patients with either psittacosis or lymphogranuloma venereum.

Complement fixation test

The most useful laboratory procedure for diagnosis of this disease is the standard complement fixation test. Virus suspensions or extracts from infected yolk sacs are used as antigen. As in the Frei test, control tests using normal yolk sac antigen prepared in the same manner as the virus antigen should be made.

To perform the test, the antigen should be adjusted to give definitely positive fixation with known positive sera and no anticomplementary effect. Sera should be inactivated immediately before use, and twofold dilutions beginning at 1:2 prepared in saline. All reagents and sera are used in 0.2 ml. amounts. The antigen and 2 units of complement are added to the serum dilution. Following incubation in the water bath for 75 minutes at 37 C., 0.2 ml. of 3 per cent sheep cells sensitized with 2 units of hemolysin is added. Readings are made following an additional incubation at 37 C. for 30 minutes. Control tubes with normal yolk sac antigen and the usual complement and antigen controls should always be included[7].

The specificity of this test also was challenged by Knott and associates[10], who found numerous patients with a complement fixation titer with no history of infection. False positive tests were reported to be especially

prominent in persons with early syphilis. The sera of these patients will usually react with both normal and virus antigens, however, as will the sera of a small number of individuals with apparent sensitivity to various constituents of normal yolk sac. Positive complement fixation tests are significantly more common in Negro patients with syphilis than in white patients with syphilis[1]. If the test is nonspecific in the presence of syphilis, comparable nonspecific reactions should occur, it seems, regardless of race.

In a study by Shaffer and Rake[2] similar conclusions were reached. In a group of children with non-venereal gonorrhea, none were found to give a positive lymphogranuloma venereum complement fixation test, whereas 9 of 19 adults with venereally acquired gonorrhea gave a positive test. Only 2 of 34 persons with congenital syphilis showed evidence of lymphogranuloma venereum infection, whereas 57 of 97 persons with acquired syphilis did show such evidence.

In an informative study by Wall, Heyman, and Beeson[3], 27 patients with proved lymphogranuloma venereum infection and 45 patients with proved chancroid were studied. Over one half of the latter patients showed complement fixing antibodies for lymphogranuloma venereum, although in low titer. All of the patients with lymphogranuloma venereum showed titers within the range of 1:40 to 1:640. Approximately one year after infection, however, the titers found in these patients were comparable to the low titers found in the group with chancroid.

The complement fixation test may be negative in early infection, and considerable information can be obtained by repeated tests[11]. There is increasing evidence that greater specificity is possible when quantitative tests are performed. Dulaney and Packer[13] conclude that titers of 1:40 or greater indicate active infection and that titers of 1:5 may be nonspecific. Similar conclusions have been reported by others[6,12]. The technique of Bowser and Nigg[14] of preparing boiled phenol-treated supernatent antigens reduces the nonspecific reactions seen with sera from patients with syphilis.

The antigens used in the lymphogranuloma venereum complement fixation test do react specifically with sera from patients with psittacosis and similar infections. Attempts to prepare specific antigens for lymphogranuloma venereum which do not cross-react with such sera have been reported[12]. At least two complement fixing antigens are present in the elementary bodies of these viruses[15]. The heat stable antigen reacts almost equally well with sera against any member of the group. A heat labile antigen with apparent specificity is also present. When sera of patients with lymphogranuloma venereum infections are absorbed with heated suspensions (group antigen) of either psittacosis or lymphogranuloma venereum virus, and subsequently tested by the complement fixation technique using fresh unheated virus suspension (specific and group antigen) as antigen, specific results are obtained and cross reactions do not occur.

Summary

1. Lymphogranuloma venereum is a common infection, particularly in Negroes, and may occur with a minimum number of early symptoms.

2. The specific laboratory procedures used in the diagnosis of lymphogranuloma venereum have been reviewed and evaluated.

3. Available evidence indicates that the skin test and complement fixation test are specific and sensitive procedures for diagnosis.

4. Standard procedures and experimental techniques for the performance of these tests have been reviewed.

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ON THE PATHOGENESIS OF OBESITY

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It is axiomatic that in any argument one must define his terms. In common usage, the word "obesity" means overweight, but this concept is unsatisfactory for two reasons: (1) it implies knowledge of an ideal weight which is exceeded; and (2) a person may be overweight and yet not obese, as in severe edema. In a more exact sense, obesity refers to the presence of an excessive amount of adipose tissue. Brozek and Keys[1] have recently pointed out that persons of "normal" weight according to age-weight tables may differ considerably in body composition; this is strikingly seen in the relative increase in body fat content at constant weight in older persons. Hence the best estimate of obesity is one based on an actual calculation of the amount of adipose tissue in the body.

The Measurement of Obesity

Several methods have been devised for measuring the fat content of the body. Talbot[2] suggested that the creatinine coefficient (milligrams of creatinine excreted in the urine per kilogram of body weight per 24 hours) which measures total muscle mass may be used as an indirect index of body fat, since increase in fat content depresses the creatinine coefficient. This method is extremely simple, but it has not been evaluated against more direct methods of measuring fat content. It does, however, have a potentially useful application in the determination of the "ideal" weight for purposes of calculating basal metabolic rate in obese persons (Tager and Kirsch[3]).

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The first really quantitative technique for the measurement of body fat content was described by Behnke, Feen, and Welham[4] in 1942. This was based on the inverse relation between fat content and body specific gravity, the latter determined by Archimedes' principle from body weight in air and under water. The validity of this method was established by comparison with the direct extraction and chemical determination of total body fat in guinea pigs (Rathbun and Pace[5]). It was also established (Pace and Rathbun[6]) that total body water is a constant fraction of fat-free body mass; fat is considered to act as a diluent of body water, and hence the two are inversely related and either may be used to calculate the other. More recently, methods have been perfected for the accurate measurement of total body water in man by the use of antipyrine (Soberman and colleagues[7]) and of deuterium oxide (Schloerb and others[8]), and satisfactory agreement has been demonstrated between values for body fat content in man derived from body water and body specific gravity measurements (Osserman and others[9]).

Unfortunately, neither the body specific gravity technique nor the body water technique for estimating obesity is sufficiently rapid and simple for routine clinical use. They do, however, provide useful research standards by which to judge the usual clinical criteria of obesity, such as height-weight relations, as well as the hoped-for quantitative clinical methods of the future.

Since the clinical evaluation of obesity is based largely on the relation between height and weight as given in standard tables, it may be pertinent to examine the validity of these tables. They are based on the *average* weights of large numbers of men and women for each height and age group; this method of computation ignores the very high incidence of obesity in the older age groups and results in "normal" weights which are too high. If it be granted that the "ideal" weight of a person at any age is that weight which results in the longest life expectancy, tables of "ideal" weight may be constructed; these differ considerably from the "average" weight tables. It has been shown (see Rynearson and Gastineau[10] for references) that "maximal life expectancy is obtained if the average weight at ages 25 to 30 years is maintained the remaining years of one's life." It is to be hoped that estimations of

obesity from height-weight tables will, in the future, be based on tables of ideal rather than average weight. (These tables may be obtained from the Metropolitan Life Insurance Company.)

The Medical Importance of Obesity

The incidence of obesity in the general population has seldom been appreciated. Approximately 28 per cent of all people are 10 per cent or more overweight; between the ages of 40 and 60, approximately 60 per cent of all women and 35 per cent of all men are overweight (Rynearson and Gastineau[10]). This makes obesity the most frequently encountered of all physical abnormalities.

The handicaps imposed by obesity are too well known to require elaboration. It is perhaps worth while to point out, however, that by the correction of obesity, the physician may ameliorate considerably the effects of many diseases for which otherwise he has little really effective treatment. The overall medical importance of obesity is best appreciated from the statistical data presented by Newburgh[11] (1942), which indicate that for the age group 45-50, the death rate increases roughly 1 per cent for each pound of excess weight.

The Physiology of Obesity

The nature of adipose tissue

Under normal nutritional conditions, fat constitutes from 10 to 15 per cent of body weight. Every cell in the body contains some fat as an integral part of its structure, and this structural fat varies little with nutritional state. Certain tissues, however, have the capacity for storing large amounts of fat in relatively inert form. These are the adipose tissues, and a knowledge of the factors which regulate the deposition and mobilization of fat in these tissues is fundamental to an understanding of the nature of obesity.

Virchow taught, many years ago, that adipose tissue is simply ordinary connective tissue, and that fat may be stored wherever connective tissue occurs. More recently this concept has been challenged (Wells[12]) with the suggestion that adipose tissue is a separate organ, a part of the reticulo-endothelial system which is derived, via the lipoblast, from primitive mesenchyme and not from pre-existing fibroblasts.

It has been demonstrated (Wertheimer and Shapiro[13]) that much of the food fat, after absorption from the intestine, is deposited directly in adipose tissue, and that penetration of fat into the adipose cells is an active process, depending on cellular metabolism and inhibited by cyanide and fluoride. Furthermore, the deposition of fat in adipose tissue is not regulated by the blood fat level, since the latter is elevated during the mobilization of fat in starvation.

The conversion of carbohydrate to fat occurs in adipose tissue as well as in liver, and involves the intermediary formation of glycogen in the fat cells (Wertheimer and Shapiro). This conversion occurs even though liver glycogen stores are not loaded, and requires the action of insulin.

Numerous workers have demonstrated the innervation of adipose tissue by sympathetic nerve fibers (see Wertheimer and Shapiro[13] for references); and Hausberger (quoted by Wertheimer and Shapiro) found that denervated adipose tissue accumulated fat more rapidly than did normal adipose tissue, apparently indicating a tonic inhibitory effect of the nervous system on fat deposition. It is interesting to speculate on the bearing of these experiments on the mechanism of the obesity resulting from hypothalamic lesions.

As might be expected, the endocrine system has also been implicated in the control of fat deposition and mobilization. Reiss[14] has reported the mobilization of stored fat by the pituitary lactogenic hormone in the rat, and preliminary clinical trials on obese patients have been encouraging. Adrenalectomy has long been known to inhibit the deposition of fat in both liver and fat depots, but Ingle[15] claims that any influence of the adrenal gland on fat metabolism is indirect, involving alteration in the conversion to fat of glucose derived by gluconeogenesis from protein.

It has long been debated whether or not the excess adipose tissue in the obese is metabolically inert. This question is of more than academic interest, since it vitally influences our calculation of the true basal metabolic rate in obese persons. Strang and Evans[16], and Short and Johnson[17] believe that the excess adipose tissue is metabolically inert and that basal metabolic rate values calculated on the basis of actual body weight are therefore too low in obese subjects, giving rise to a false impression of hypome-

tabolism. Talbot and others[18], and Mirski[19], on the other hand, believe that adipose tissue is an active organ with a specific carbohydrate metabolism, a concept supported by the isotope studies of Schoenheimer[20].

Basal metabolism in obesity

Since the level of the basal metabolic rate in obese persons has an important bearing on current concepts both of the etiology and of the treatment of obesity, it is a topic which warrants careful consideration. Two interrelated questions must be answered: (1) How valid is the expression of the metabolic rate in terms of an empirically calculated body surface area? (2) Does the surface area law apply without modification to obese persons? It must be confessed that too often these questions have been answered more by faith than by logic. The concept that basal metabolism is proportional to body surface area has been developed by a process of reasoning involving the following steps:

1. Both metabolic rates per unit weight and body surface area per unit weight diminish with increasing body size.

2. Heat loss from an object is proportional to its surface area.

3. If a constant body temperature is to be maintained, heat production must balance heat loss and hence also be proportional to surface area.

This line of reasoning has led to the assumption that body surface area actually determines heat production, a proposition which is difficult for me to accept. Why, for example, should the heat produced by a liver cell in its daily activity be greater in a lean person with high relative surface area than in an obese person with low relative surface area? As a matter of fact, basal metabolic rate in animals of varying sizes is more nearly proportional to the three-fourths power of the body weight than to the two-thirds power of the body weight to which surface area is proportional (Kleiber[21]). In this connection, the recent experiments of von Bertalanffy and Pirozynski[22] are of interest. These workers reported that the Q_{O_2} values of the major organs and tissues of albino rats ranging in weight from 9 Gm. (newborn) to 392 Gm. (adult) did not diminish in accordance with the surface area law nor with the weight three-fourth factor recommended by Kleiber. They suggest that decreasing metabolism with increasing body weight is due to factors involving the organism as a whole, not to decreasing metabolism of each cell.

The second question posed is no less difficult to answer. When measured in the conventional manner, the basal metabolism of obese persons is usually within normal limits. If, however, the excess adipose tissue has a metabolic rate significantly lower than that of the other body tissues, as seems likely, the "metabolically active mass" of the obese person must actually have a supernormal metabolic rate. The possible dangers of the use of thyroid for weight reduction in such a situation are obvious.

Actually, the true metabolic rate in obese persons has not been satisfactorily determined. The validity of the clinical basal metabolic rate measurement is open to question, and protein-bound iodine determinations have given equivocal results (Williams[23]). This problem is currently being investigated in the writer's laboratory.

Regulation of body weight

It is a matter of common observation that most adults tend to maintain a reasonably constant body weight year after year despite daily variations in caloric expenditure. Temporary fluctuations in body weight incident to enforced inactivity or to inanition are usually self-limited and quickly reversed when the abnormal stimulus is removed. This implies the existence of a homeostatic regulation of body weight comparable to that which maintains the constancy of the blood sugar, body temperature, and other features of the internal environment. Obesity may be considered to be the result of a perversion of this homeostatic mechanism, just as are the hyperglycemia of diabetes mellitus and the hyperpyrexia of infectious diseases. It is self-evident that until the mechanism which normally maintains a constant body weight is better understood, all attempts to explain the basic causes of obesity will be largely empirical, and the treatment equally so.

Most of the physiologic studies on hunger and appetite have been concerned with the nature of the stimulus which leads the organism to seek food. It is no doubt true that the oral gratification of neurotic needs by the act of eating is responsible for many cases of obesity. However, a matter of equal importance, which has been largely neglected, is the factor which determines satiety.

In this connection, the experiments of Strang and his associates[24] are outstanding. They demonstrated that there was no difference in the specific dynamic action of a standard test meal in normal, obese, and thin subjects. However, the time course of the increased heat load was quite different, being delayed in the obese subjects. Measurements of skin temperature revealed a significant fact: normal weight subjects showed a greater and more prompt elevation of skin temperature during and after a meal than did obese subjects. Persons of normal weight were unable to continue eating with a rise in skin temperature of 0.9 C. in 22 minutes. The smaller rise in skin temperature of the obese prevented the attainment of the level at which the normal people could eat no more. This phenomenon might well be related to the slower rate of change of heat production of the obese in response to ingested food, and may account for the consumption of food by the obese in excess of the actual physiologic requirements. Experiments of this type indicate that real differences may exist in the mechanism which determines satiety in normal and obese persons and help to explain the frequent failure of permanent weight reduction by dietary restriction alone.

Daily variations in weight are much greater in obese than in normal persons, due largely to greater variations in the water content of the body. The body heat regulating mechanism is disturbed under the conditions imposed by obesity; with changes in environmental temperature and humidity, larger volumes of water exchange take place. Furthermore, fat deposits have a variable capacity for water storage, said by Lauter[25] (1926) to range from 7 to 70 per cent. This variable capacity of fat tissue for storage of water is of importance in relation to the observed weight changes in obese subjects undergoing weight reduction.

A final point of physiologic interest is the self-limited nature of the weight gain in obesity. It was thought at one time that even a very small daily excess of food would lead to unlimited weight gain, and the maintenance of a constant body weight was attributed to an appetite-controlling mechanism of incredible accuracy. The fact that increase in body weight results, *pari passu*, in an increase in caloric requirement was overlooked. Gastineau and Rynearson[26] have calculated that an increase in body weight from 150 to 200 pounds increases the caloric requirement for moderate activity from 2415 to 2760. This means that a 150 pound man eating a 2760 calorie diet would stop gaining weight when he achieved a weight of 200 pounds. Conversely, on a daily caloric intake of 2415 calories, our 200 pound man would lose weight until a new balance was reached at a weight of 150 pounds. This simple caloric book-keeping is the basis of weight reduction by caloric restriction, and it is invariably successful when it is used correctly.

Theories of the Etiology of Obesity

It was popular practice at one time to classify obesity as either exogenous or endogenous. Exogenous obesity was considered to result from consumption of food in excess of requirements and consequently to be corrected by simple dietary restriction. Endogenous obesity, on the other hand, was a somewhat mysterious condition whose victim was doomed by hereditary, constitutional, or endocrine abnormalities to a lifelong state of obesity even though caloric intake was not excessive. In spite of the overwhelming evidence that all obesity is exogenous, in the sense that it is due to an excess of caloric intake over caloric requirement, a casual perusal of current medical journals indicates that the concept of endogenous obesity still has many supporters. Following the classification proposed by Rynearson and Gastineau[10], most of the "endogenous" causes of obesity may be grouped under one of three headings: (1) endocrine factors, (2) genetic factors, and (3) disorders in the use of energy.

Endocrine factors

In the early years of this century, Fröhlich described a syndrome of obesity and genital dystrophy which he attributed to hypopituitarism. Even though later work demonstrated that the hypothalamus, not the pituitary, is the site of the abnormality in Fröhlich's syndrome, the concept of "pituitary obesity" has died slowly. Cushing's syndrome is the one variety of obesity which is associated with pituitary disorder, and even in this instance adrenal cortical hormones probably mediate the metabolic changes responsible for the characteristic distribution of adipose tissue.

It is commonly but erroneously believed that myxedema and hypothyroidism without

myxedema are usually associated with obesity (Bonime[27], and Goldzieher[28]). However, well controlled animal experiments (MaKay and Sherrill[29]) and statistical studies on patients (Plummer[30]; Seward[31]) have shown that hypothyroidism does not tend to cause obesity. Apparently the appetite is diminished in proportion to the decreased level of caloric expenditure.

Proponents of the endocrine theory of obesity invariably refer to the "characteristic obesity of Cushing's disease." There seems to be little doubt that in this condition the adrenal cortical hormones have modified the normal distribution of fat, though the obesity may be more apparent than real, owing to weakened abdominal muscles and shortening of the trunk and neck by osteoporosis and flattening of the vertebral bodies (Albright[32]). The total amount of adipose tissue is not invariably increased in Cushing's disease (Ingle[15]) and patients with Cushing's disease lose weight on a reduction diet just as do other patients (Freyberg and Newburgh[33]).

It is almost universally believed that hypogonadism is associated with obesity—in fact, a thin eunuch would be regarded with distinct suspicion! However, despite the almost invariable obesity which follows gonadectomy in animals, there is no proof that hypogonadism in the human is associated with increased incidence of obesity (Greene[34]; Silver and Bauer[35]). It has been pointed out that the onset of obesity often coincides with periods of gonadal change, such as puberty, pregnancy and the menopause, and this has been taken as evidence for an endocrine basis of the obesity. However, the shifts in hormone levels are not even in the same direction in these diverse situations, and the psychic conflicts so common at these times are a more likely cause of overeating.

Genetic factors

The Laurence-Moon-Biedl syndrome, consisting of mental deficiency, retinitis pigmentosa, hypogenitalism, obesity, and polydactyly, appears to be inherited as a recessive character. It seems likely (Warkany[36]) that this syndrome is the rare, chance combination of relatively frequent heredofamilial anomalies. There is no proof that the obesity results from any factor other than a large appetite and decreased activity.

The Morgani - Stewart - Morel syndrome (hyperostosis frontalis interna) includes internal hyperostosis of the frontal bone, headache, and frequently obesity, sexual disturbances, hypertension, and psychoneurotic tendencies. The obesity again seems to be the result of derangement of the appetite-regulating mechanism.

Obesity which occurs frequently in certain families but without other congenital abnormalities is often regarded as truly familial in nature. Bauer[37], in particular, has championed the idea of an inherited, abnormal avidity of the adipose tissue for fat ("lipophilia") which, by depriving other parts of the body of nourishment, increases hunger and hence the intake of food. According to this view, the increased appetite is the result, not the cause of obesity. There is no experimental evidence to support this concept, and much to oppose it.

Among the poorly understood conditions associated with obesity, one may mention progressive lipodystrophy and adiposis dolorosa (Dercum's disease). In both conditions, dietary restriction results in loss of fat from the regions of obesity.

Disorders in the use of energy

A common complaint on the part of obese people is that they remain fat in spite of very modest caloric intakes. However, in every case in which the caloric value of their diets has been checked it has been found to be high, and weight loss has proceeded according to calculation under controlled dietary restriction (see Newburgh[11,38] for references).

Among the specific energy abnormalities which have been claimed to cause obesity, together with discrediting references, are the following: excessive gastrointestinal absorption (Wilder[39]), increased efficiency in doing work (Newburgh[38]), and reduced specific dynamic action of foods (Strang, McClugage, and Evans[40]).

In summary, there seems to be very little evidence to support any of the numerous endogenous factors which have been suggested as causes of obesity. It would appear that in every case obesity is actually exogenous in origin—that is, the result of excessive intake of food. This simplifies, but by no means solves, the total problem of obesity by focussing attention on the cause and correction of the increased food intake.

Emotional Aspects of Obesity

(Valuable source material on this topic may be found in Richardson[41] and Hamburger[42]). It is common knowledge that when a person is upset or under some emotional tension there is often a reflection in his appetite, which may be either increased or decreased. "In morbid emotional states, particularly the depressions, eating disturbances are usually cardinal symptoms. Often the neurotically depressed person will overeat and gain weight, whereas the psychotically depressed person often refuses food. Thus in sickness and in health there is an intimate interrelationship of appetite and the person's emotional state."[42] Despite these leads, the emotional basis of obesity has only recently begun to receive serious attention. The failure of many obese patients to persist in weight-reduction programs as well as the realization by the patients themselves that they overeat when emotionally aroused have been largely instrumental in bringing about this reorientation of thinking on the part of physicians. For example, Freed[43] asked 500 obese patients, "When you are nervous or worried do you eat more or less?" Only 35 of the 500 patients reported no increase in food intake under conditions of anxiety or of boredom.

This reaction in obese persons does not appear to differ qualitatively from that of normal people, but quantitatively their appetite response to an emotional stimulus is greater than that of the healthy person.

Overeating as a response to nonspecific emotional tensions

Hamburger found that 12 of his series of 18 patients whose obesity was on an emotional basis were aware of marked changes in appetite accompanying transient, nonspecific emotional upsets. Two of these ate less and the other 10 ate more. It is of interest in connection with the routine questionnaire method that some patients reveal a relation between appetite and emotional tension only after many hours of psychotherapeutic reviewing.

Overeating as a substitute gratification in intolerable life situations

Seven of Hamburger's patients gave a history of reacting to intolerable or frustrating life situations with chronic overeating. These patients apparently gained some sort of substitute emotional gratification and relief of tension by overeating.

Overeating as a symptom of an underlying emotional illness, especially depression and hysteria

Eight of Hamburger's patients developed hyperphagia as only one of many symptoms of an underlying emotional illness. Of the entire series of 18 patients, 12 exhibited depressive features in their histories, and 3 had made suicidal attempts. Others became depressed as they lost weight under treatment; this has been noted by other workers, and has no doubt been responsible for the frequent abandonment of attempts at weight reduction. Six of Hamburger's patients, all hysterical women, overate in response to sexual conflicts, either real or imagined. For various reasons these women failed to mature emotionally, and were incapable of enjoying an adult sexual life. The psychoanalytic explanation would be that since these women could not accept genital gratification, they substituted the oral gratification of eating. To them eating had an unconscious sexual significance. The fact that overeating leads to obesity, which diminishes the chances of heterosexual contact and responsibilities, is probably also a factor of some importance in these immature women.

The foregoing section is not intended to be a complete analysis of the emotional basis of obesity, which is beyond the scope of this paper. It is presented rather as an illustration of the complex psychologic factors underlying obesity in one thoroughly studied series of patients. It is not possible to say what proportion of cases of obesity has basically a psychologic etiology, but it must be very large. Failure to consider this factor has frequently been responsible for disappointing results in weight-reduction programs.

General Principles in the Treatment of Obesity

The treatment of obesity is very simple in theory, but it may be extremely difficult in practice. If it be granted that all obesity is caused by a caloric intake which exceeds caloric requirement—that is, by overeating— it follows that the logical treatment is caloric restriction. Newburgh (*loc. cit.*) has demonstrated conclusively that obese patients always lose weight on a restricted diet, if allowance is made for the water

retention which often occurs during the early weeks of treatment. An excellent discussion of the dietary management of obesity is found in Rynearson and Gastineau's monograph[10].

Psychotherapy

It should be clear from the discussion of the etiology of obesity, that dietary restriction will often be doomed to failure unless the basic factor responsible for overeating is corrected. Superficial psychotherapy in the form of encouragement and reassurance may be all that is needed. Some patients will adhere to their diet only if the possible dangers of obesity are clearly stressed. Where overeating serves a deep seated neurotic need, more extensive investigation and psychotherapy are necessary. Moreover, obese neurotic patients often become depressed during dietary restriction, especially if an attempt is made to reduce the weight too rapidly. These patients require expert guidance; dietary restriction without psychotherapeutic reassurance is almost never successful.

Drugs

Two types of drugs have been used as adjuncts to dietary restriction in the treatment of obesity. These are the anorexigenic drugs, typified by amphetamine, and the metabolism-stimulating drugs, typified by thyroid extract.

Amphetamine alone may bring about weight loss as a result of depression of the appetite and a voluntary reduction in food intake. Harris and Ivy (quoted by Rynearson and Gastineau[10]) demonstrated in animal experiments that the anorexia induced by amphetamine is of central origin and independent of any effect on gastric motility or secretion. The pressor and stimulant side-effects of amphetamine led to a condemnation of its use as an adjunct to weight reduction by the A.M.A. Council on Pharmacy and Chemistry; more recently the Council has withdrawn its objection, provided the drug is used under careful medical supervision. It is generally accepted that amphetamine should not be used as a substitute for, but rather as an adjunct to planned dietary restriction. It is particularly useful when the weight loss begins to decelerate after some weeks of dietary restriction (Douglas[44]).

There is considerable difference of opinion concerning the advisability of using thyroid in the treatment of obesity. It has already been pointed out that hypothyroidism is rarely associated with obesity and that most obese persons may actually be hyperthyroid if calculations are based on true "metabolic weight." Small doses of thyroid are probably inactivated in euthyroid subjects (Winkler and others[45], 1943), large doses are effective only by causing hyperthyroidism (Means[46], 1935), and its associated dangers, especially damage to the heart, must be borne in mind. The majority opinion today favors the use of thyroid extract only when definite clinical signs of hypothyroidism are present.

Diuretics

Newburgh (*loc. cit.*) has emphasized that reduction of adipose tissue by dietary restriction may induce water retention which often obscures the expected weight loss. There is usually a spontaneous diuresis during the early weeks of treatment, so that diuretics are seldom needed. Pelner[47], however, believes that most patients require diuretics for optimum results. If diuretics are to be used, it is suggested that the less harmful ones, such as ammonium chloride and urea, be employed. The restriction of salt in the diet serves the same general purpose, but the restriction of water serves no useful purpose. Similarly, loss of weight in heat cabinets resembles merely loss of water as sweat, and the deficit is soon made good.

Exercise and massage

These are popular but ineffective methods of weight reduction. A rather tremendous amount of exercise is required for weight reduction, and this is often dangerous to the already oxertaxed heart of the obese person.

Massage is even less effective (Kalb[48], 1944; Short and Currence[49], 1939) in mobilizing either generalized or localized deposits of fat.

Summary and Conclusions

1. Obesity is the most common physical defect found in the general population.

2. Correction of obesity significantly increases longevity and decreases morbidity and mortality in a wide variety of disease states.

3. Adipose tissue is a specialized tissue with its own metabolism, not merely connective tissue which passively stores fat.

4. The basic factor in all types of obesity is excessive food intake.

5. Endocrine, genetic, and metabolic abnormalities account for a small proportion of cases of obesity.

6. In most cases of obesity, increased food intake is the result of psychosomatic needs.

7. The basic principle in the correction of obesity is dietary restriction. Psychotherapy of some type is usually necessary for successful dietary management, and drugs may at times be useful adjuncts.

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THE PLACE OF CYSTINURIA IN HUMAN DISEASE

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Cystinuria, or the urinary excretion of abnormal quantities of cystine, has been regarded for many years as an "inborn error of metabolism," rare and comparatively harmless. In common with other amino acids, cystine is excreted in normal urines to the extent of 1 to 10 mg. per day. In the cystinuric this output may reach 1 or even 2 Gm. per day. Even in cases of severe cystinuria the condition is not highly objectionable, and a cystinuric subject may live a normal life except for the complications arising from the liability, often realized, of continued calculus formation in the urinary tract. This

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liability is due to the fact that cystine is one of the very few amino acids whose solubility is extremely slight when in the free state. Its minimum solubility occurs at about *p*H 4.0, and it is only slightly more soluble at the normal urinary *p*H of 6.0. In more alkaline urines (*p*H 7.0 to 7.5), its solubility is much increased, owing to formation of the soluble salt, a circumstance of which clinical advantage can be taken. The constant and lifelong liability to the cystinuric is therefore that of calculus formation in kidney or urinary bladder. This fact, together with the ease with which cystine stones are often confused with those of other composition, creates a situation justifying far more clinical attention than has usually been accorded it.

Although cystinurics are rare, they are not as rare as has been claimed. The best available data[1] indicate that its incidence is from one in 500 to one in 1000 persons. While many of these patients excrete the cystine harmlessly in solution because of colloidal materials in the urine which prevent crystallization, many also deposit "gravel" or calculi; and the possibility of the latter is a lifelong threat. From the standpoint of the cystinuric patient, and to distinguish him from other calculus-formers, an accurate diagnosis is essential.

Although one of the first amino acids to be described in the literature, cystine was not recognized as a normal constituent of proteins for nearly a century later. For its early discovery we may thank the nameless cystinuric subject whose calculus was sent to Dr. W. H. Wollaston. Dr. Wollaston published, in 1810, a brief account of the properties and composition of this substance, which he named "cystic oxide," a name subsequently shortened to cystine. In 1899, Morner, the Swedish investigator, reported the presence of a form of cystine in proteins, and after about 30 years more it was generally agreed that the two forms, that in calculi and that in proteins, are identical.

Up to about 1921 cystine was regarded as the only sulfur-containing constituent of proteins, and all sulfur present in the latter was credited as representing cystine. At this time, however, J. H. Mueller reported the presence in a number of proteins of methionine. Previously, cystine was classed as one of the amino acids essential to growth. Further investigation, once the presence of methionine was established, showed the latter to be a dietary essential with cystine occupying the place of a partial substitute. The role of methionine in metabolism has loomed greater with continued investigation. It is not only the chief source of the biologically essential methyl group, but its sulfur is convertible by the animal into cystine. It seems to be the case that the animal can convert methionine into cystine, but not the reverse. Nevertheless, cystine, while not a dietary essential, plays an important part in metabolism, and is present in nearly all body proteins, particularly the keratins.

Under normal conditions, the final metabolic oxidation product of these amino acids is sulfuric acid. Since the normal *p*H of urine does not permit the excretion of the unneutralized acid, it is actually present as a mixture of soluble sulfates, chiefly sodium sulfate. A small proportion of urinary sulfate is diverted into the form of sulfate esters as a result of a detoxication process. This fraction is commonly known as the "ethereal sulfate." A similar small amount of the urinary sulfur is excreted in a variety of unoxidized forms, among which are the minute amounts of cystine found, as stated above, in all normal urines. Under normal dietary conditions, about 1 Gm. of total sulfur is excreted per 24 hours. An average normal percentage partition of this sulfur is as follows:

Inorganic sulfate . .	90 per cent
Ethereal sulfate . . .	5 per cent
Unoxidized sulfur . .	5 per cent

In the cystinuric subject, the excretion of excessive amounts of cystine, being a defect in sulfur oxidation, greatly changes these figures. The excreted cystine, representing unoxidized sulfur, falls into that category at the expense of the inorganic sulfate. As a result, urine from a typical cystinuric would show a percentage partition somewhat as follows:

Inorganic sulfate . .	40 per cent
Ethereal sulfate . . .	5 per cent
Unoxidized sulfur . .	55 per cent

The severity of the cystinuria, however, considerably influences these figures.

From the chemical point of view, the problem of biologic oxidation of the sulfur of both cystine and methionine is most fascinating. No other chemical element metabolized by the animal body undergoes such a wide change of valence in its oxidation, a

change virtually from its lowest to its highest valence, minus 2 to plus 6. In view of this fact we might well expect to find more individuals in the world showing defective oxidation of sulfur, and therefore excreting abnormal amounts of cystine. It is a curious fact, one for which we have yet no good explanation, that cystine administered by mouth to a cystinuric subject is oxidized in the normal manner to inorganic sulfate. The cystine excreted by the cystinuric would seem to be of endogenous origin. Methionine, when fed by mouth to the cystinuric, has been reported by some investigators to produce increased excretion of cystine[2] while others[3] have failed to observe this increase. In one case, reported by the author[4], a subject who at a pre-adolescent age (13 years) showed no increased cystine excretion on methionine administration did, three years later, show a moderate increase in cystine output under these conditions. This same subject, however, showed a greater increase in cystine output as a result of diuresis after quadrupling his water intake.

The effect of the level of protein intake on cystine excretion has also received considerable attention. It is generally agreed that a high protein intake produces increased cystine excretion. By some investigators this is ascribed to the methionine content of the protein[2a], whereas others have reported comparable increases resulting from administration of even some of the amino acids (glycine, alanine, and so forth) containing no sulfur[3b]. It is obvious that the exact mechanism of this abnormality is still obscure.

The most obvious clinical manifestation of cystinuria is the formation of calculi in the kidney or urinary bladder. These have been known to reach considerable size. Tennant[7] reported the removal from a patient of several cystine stones, the largest of which, from the cortex of the right kidney, weighed 50 Gm. Other cystine calculi of similar size have been reported. As far as present records show, no calculus deposited by a cystinuric has any other composition than that of practically pure cystine. An alkaline ash diet, producing a urine of *p*H 7 or above, can materially aid in dissolving small cystine calculi or "gravel," whereas precisely the opposite treatment (an acid ash diet) is indicated for the most common of all forms of calculi: tricalcium phosphate.

The importance to the cystinuric patient of a correct diagnosis of his situation is obvious when it is realized that an acid ash diet, involving a high level of protein ingestion, not only increases the cystine output by reason of the larger amounts of protein metabolized, but also renders the cystine less soluble by reason of the lowered *p*H of the urine. It should again be emphasized that many cystinurics do not deposit even small calculi; but for any such subject such stone formation is a lifelong liability. There are no known cases in which the condition has been cured: Once a cystinuric always a cystinuric. Perhaps no one fact about this condition is so well established as its lifelong permanence.

Cystinuria is hereditary in nature. Although complete data in this regard are none too abundant, several family trees of cystinuric families have been reported. One of these is quoted by Lewis[1]. I have reported others[6]. In the last study, Dr. Brooks and I had the opportunity to study four generations of a cystinuric family. Of the 25 members examined, 7 were cystinurics. We appear to be dealing with a recessive characteristic.

One further observation of a somewhat qualitative nature might be mentioned. In the writer's experience cystinurics are, in general, persons of somewhat less than average stature. This has been the case with all subjects personally observed, and was particularly marked in the siblings of the third generation in the family cited above[6b]. While not to be described as dwarfs in any sense of the word, the cystinurics have always been of somewhat slight build.

The therapeutic measures to be recommended in dealing with this condition may be described as follows: A prerequisite in instituting treatment is correct diagnosis. In my opinion, any case of real or suspected calculus formation should be tested. The test may be applied to any calculus or to the subject's urine. The latter will always show an abnormal content of cystine if the subject is a true cystinuric, independent of any evidence of caculus formation. The speed and simplicity of the nitroprusside test for cystinuric urines, as described by Brand, Harris, and Biloon[7] makes possible very prompt testing of either calculus or urine samples. Positive results on a urine sample call for further confirmation, but negative results can be regarded as unequivocal.

The nitroprusside test may be made as follows: To about 5 ml. of urine in a test

tube add an equal volume of a 5 per cent sodium of cyanide solution. Allow to stand about five minutes at a room temperature, then add 3 or 4 drops of a freshly prepared 5 per cent solution of sodium nitroprusside. A brilliant purple color is produced in the presence of cystine. Compare with a known normal urine.

If a patient with a correctly diagnosed case of cystinuria is depositing small calculi or "gravel," a beneficial effect may be obtained by employing those factors which make for a more alkaline urine, since at a *p*H of 7.0 or 7.5 the formation of the soluble sodium salt is markedly increased. Such factors are (1) a low protein intake, (2) the inclusion of a preponderance of "alkaline ash" dietary constituents (vegetables and most fruits), and (3) the administration of such alkalies as sodium bicarbonate or sodium citrate. The low protein intake, as explained above, serves the dual purpose of reducing the cystine output as well as of providing a more alkaline urine. If deposits of cystine are already present in the urinary tract, the sudden production of more alkaline urine will cause an increased excretion of the dissolved amino acid owing to the increased solubility. This finding has sometimes been ascribed erroneously to an increased cystine production on the part of the organism, whereas it is simply a temporary flushing-out process.

The process of alkalization of the urine is obviously favorable to the dissolving of certain other calculi, notably those composed of uric acid. On the other hand it is definitely contraindicated in oxalate calculi, and in the most common case of all—calcium phosphate. The application of alkali therapy in the latter two conditions would obviously be favorable to the further building up of calcium oxalate and calcium phosphate calculi.

Although cystinuria is regarded as a rarity, the importance to the cystinuric patient of a correct diagnosis is paramount.

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THE RELATION OF SOME RECENT BIOCHEMICAL INVESTIGATIONS TO DISEASES OF INTERMEDIARY METABOLISM

CARL E. ANDERSON, PH.D.

CHAPEL HILL

During the past several decades there has been established a considerable body of information which, though it may change considerably in detail, presents a pattern whereby the major foodstuffs and metabolites may be traced through the principal pathways of intermediary metabolism. Admittedly, the pattern, which is presented in its most general outline in figure 1, is still incomplete. There are steps in which the facts are scanty and the evidence contradictory. In some instances reactions first have been observed in microorganisms, and later found to occur as essential processes in animal metabolism. Nevertheless, in its broad aspect, the pattern is generally accepted as representing the pathway for the intermediary metabolism of carbohydrate, fat, and protein in the human organism.

It is not the purpose of this paper to review the complex field of intermediary metabolism or, for that matter, even a narrow segment of this complex structure. Rather, it is hoped to show by a few examples how recent laboratory investigative work has revealed certain information concerning fundamental metabolic patterns in the animal organism, and how through such an increase in our knowledge it is possible to obtain a clearer insight into the underlying biochemical fault of metabolic disease. In some instances the information presented here has been amply substantiated, in other instances confirmation is necessary, and in

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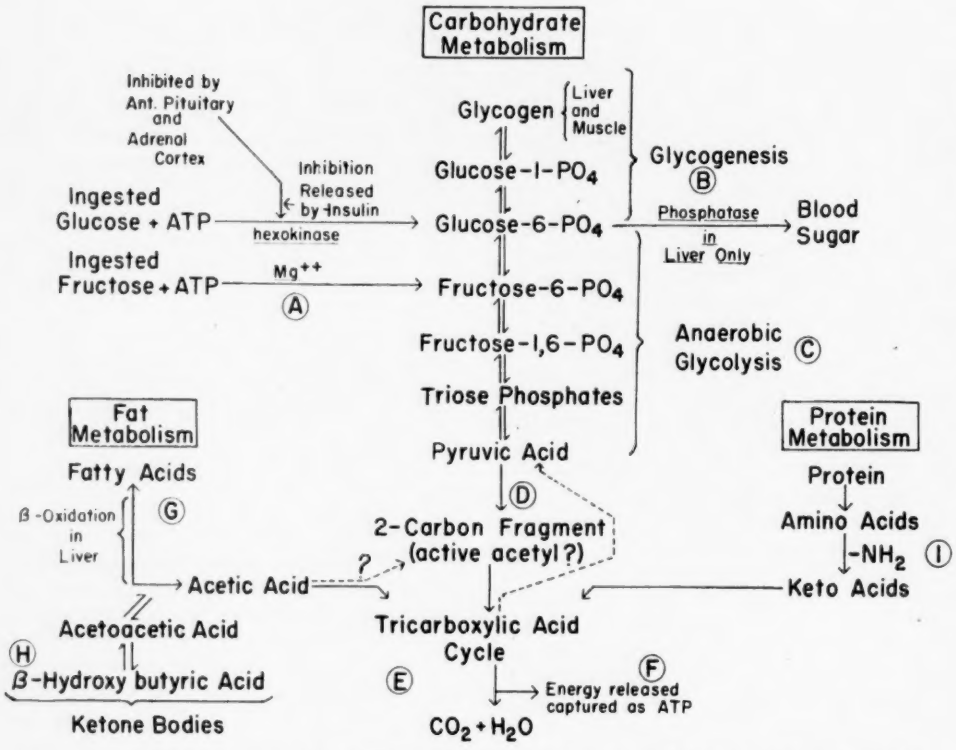

Fig. 1. Schematic representation of the metabolic pathways for carbohydrate, fat, and protein.

at least one instance the hypothesis advanced is admittedly speculative.

The "Metabolic Pool"

Since 1946 great strides in our knowledge of the processes of intermediary metabolism have been made possible by the use of isotopes or tracers, and the application of tagged molecules to established classical methods of experimentation. Such studies have revealed that previously accepted concepts of the independence of the various foodstuffs or major metabolites and a sharp separation of exogenous and endogenous metabolism are no longer possible. Instead, the metabolites arising from ingested carbohydrate, fat, and protein coverage in common 2-carbon fragments (D in figure), for which the exact mechanisms of formation and structure are still uncertain and controversial. However, through such a scheme as that shown in the figure, ingested carbohydrate, fat, and protein, initially vastly different in chemical constitution and properties, are converted by enzymatic action into a limited number of small molecules, indistinguishable in regard to their point of origin and parent compound, which then constitute a common "metabolic pool" in equilibrium with the tissues of the organism[1]. Through such a common "metabolic pool" it is possible for the animal organism to convert rapidly, by a reversal of the enzymatic reactions concerned, one foodstuff to another as needed for tissue repair or some other vital function.

The concept of a "metabolic pool," as described by Bloch[2], may not correspond to any physical reality in the sense that there exists in the body at a given moment a large reservoir of metabolites in which molecules derived from dietary and various tissue constituents are distributed in random fashion and therefore are indistinguishable. It merely means that carbohydrate, fat, and protein are interconvertible in the sense that they give rise to breakdown products which lose

the identity of origin and can be utilized interchangeably for the resynthesis of any one of the three major tissue constituents.

The liberation of energy by oxidation

It appears likely at the present time that within each metabolizing cell there is a common oxidative pathway (E in figure), the so-called tricarboxylic acid cycle or Krebs citric acid cycle[3], through which the 2-carbon fragments can be swept and oxidized to carbon dioxide and water with the release of energy. By such a mechanism it is possible to visualize how the energy of the sun, utilized by the plant in the synthesis of the foodstuff molecule, is gradually released during the degradation or oxidation of the same molecule in the animal organism and used as energy for muscle contraction and motion, as electrical energy for the transmission of nerve impulses, as chemical energy for the synthesis of tissues and other vital chemical compounds essential to the body economy, or dissipated as heat. Through such a scheme the animal body, by the investment of a relatively small amount of energy for the formation of hexose phosphate from ingested carbohydrate (A in figure), realizes a dividend in a greater return of energy through the complete oxidation of the sugar molecule. This dividend in energy in turn enables the cell and the organism to continue the life processes.

The enzymes and co-enzymes

Oxidation is the chief means for the liberation of energy in the animal organism. However, the physiologic limits imposed on the sensitive and labile tissues are such that the explosive direct union of oxygen with the food metabolite is not physiologically possible or compatible with life. Such an explosive release of energy is also obviously wasteful. Therefore, in the course of the metabolic breakdown of glucose, or for that matter any of the foodstuffs, enzymes play vital roles, not only in catalyzing each individual step, but in assisting in the mild, gradual oxidation of the glucose molecule by hydrogen transfer, or more properly by electron transfer, until hydrogen can be directly united with oxygen to form water.

In this latter role, co-enzymes are essential. Co-enzymes are organic molecules associated with a specific enzyme protein. Dissociation of the co-enzyme, or prosthetic group, from the protein portion renders the enzyme inactive. Some of these co-enzyme molecules contain vitamins of the B group. Nicotinamide, for example, is a component of the di- and triphosphopyridine nucleotides, which function as co-enzymes and assist in the oxidation of hexose phosphates during anaerobic glycolysis (C in figure). Riboflavin and thiamine function in similar capacities, as for example in the oxidative decarboxylation of pyruvic acid to a 2-carbon fragment (D in figure).

These are only a few of the instances in which vitamins function as components of enzyme systems. The point to be made is that a deficiency of one or all of these vitamins, or an inhibition of their function through disease, may seriously interfere with the normal functioning of the metabolic pathways. In thiamine deficiency, for example, pyruvic acid accumulates in the body because of a diminished ability on the part of the organism to supply thiamine pyrophosphate, a requisite part of the enzyme system involved in the decarboxylation of α-ketoacids (as for example, pyruvic acid)[4]. In addition to co-enzymes, traces of certain metals such as magnesium, manganese, calcium, cobalt, iron, and such anions as chloride are frequently necessary to promote enzymatic activity.

Diabetes Mellitus as an Example of Homeostatic Adjustment

From the foregoing it is apparent that the metabolic scheme is itself a sensitive homeostatic mechanism reacting to stress placed on any one member by a suitable adjustment in the remaining members. Many of these adjustments involve enzyme systems. The symptoms associated with increased fat and protein metabolic activity in diabetes mellitus, which is initially a defect in carbohydrate metabolism, is a classic example of this homeostatic adjustment. Here, in part at least, an interference, apparently of endocrinologic origin, with an enzymatic reaction essential to normal carbohydrate metabolism stimulates an increased fat and protein metabolic activity. If, then, one way of defining life is an orderly functioning of enzymes, and if disease manifests itself as a disorder, inhibition, or hyperfunctioning of enzymes[5], it is obvious that an understanding of the normal processes of intermediary metabolism, and its many enzyme

systems, is not only of theoretic interest, but of immense practical value in understanding a disease and its treatment.

Ingested glucose and other utilizable monosaccharides are not directly oxidized by cells but enter the metabolic stream by first being converted to hexose phosphates (A in figure). In the case of glucose, phosphate is transferred to the sugar from adenosine triphosphate (ATP) or adenosine diphosphate (ADP). The latter substances are then reconstituted through the energy derived from the oxidation of glucose in the glycolytic (C in figure) and tricarboxylic acid cycles (E in figure).

The reaction transferring phosphate to sugar is catalyzed by the enzyme hexokinase. This enzyme was first discovered in yeast by von Euler and Adler[6], and independently by Lutwak-Mann and Mann[7]. The enzyme was first crystallized by Cori and his associates[8], and requires magnesium ions for the reaction to proceed. Recently, several laboratories have presented evidence for the existence of separate glucokinases and fructokinases in animal tissues[9]. Inasmuch as this reaction is an obligatory first step and must occur before glucose may be either converted to glycogen in the liver and muscles, or catabolized to yield energy, the reaction is obviously of great significance and of fundamental importance to carbohydrate metabolism.

Etiology of Diabetes

Diabetes mellitus is a disease characterized by hyperglycemia and impaired glucose metabolism. The exact etiology of diabetes in man is still unknown. Although the metabolic defect can usually be corrected by the administration of insulin, the diabetic state may be more complex than just a simple insulin deficiency. The production of experimental diabetes by the destructive effect on the beta-cells of the pancreas by alloxan, a uric acid derivative, has led to the suggestion that in human diabetes an alloxan-like metabolite is formed, possibly in the course of purine metabolism. This and other possible factors contributing to the etiology of diabetes have been recently reviewed by Lazarow[10]. Evidence to date would indicate that diabetes develops as a consequence of disturbance in the balance between insulin production on the one hand and factors modifying its utilization on the other.

Until comparatively recently the probable mechanism by which insulin acts has remained a mystery. The brilliant studies of Cori and Cori, and their associates[11] have shown that secretions of the anterior pituitary gland and adrenal cortex together act to inhibit the action of hexokinase in its role of catalyzing the phosphorylation of glucose to glucose-6-phosphate. Insulin, which apparently is without effect upon hexokinase itself, effectively blocks this inhibition. It follows, then, that an insulin deficiency or an excess of anterior pituitary or adrenal cortical hormone might inhibit hexokinase unduly and thus effectively decrease glucose utilization, which would result in hyperglycemia and failure to store glycogen. Conversely, an excess of insulin might be expected to overcome the inhibition of hexokinase, and glucose might be phosphorylated at a normal or faster rate.

On the basis of these findings, it is presumed that the diabetic state may result from either excessive secretions of anterior pituitary and adrenal cortical factors which inhibit the hexokinase reaction, or a deficient secretion of insulin which normally counterbalances the inhibition from the hexokinase reaction. The finding of Villee and Hastings[12] that glucose utilization was accompanied by glycogen deposition in all but the adrenalectomized animal, and that insulin increased glycogen formation lends support to the concept, as do the findings of other workers who have demonstrated the antagonistic action of the adrenal and pituitary hormones on the one hand, and insulin on the other.

In addition to the metabolic implications of this work is the fact that this was the first demonstration of hormone action on a biochemical level. Although the work of Cori and his associates on the hexokinase mechanism fits well with many of the facts of clinical diabetes, the hexokinase inhibition and activation may be only a part of the picture. Furthermore, a word of caution concerning its general acceptance is necessary because of some of the contradictory results obtained by Stadie and Haugaard under somewhat similar experimental conditions[13].

Relationship of Diabetes to Increased Fat Metabolism

According to the figure, the end product of the glycolytic phase of carbohydrate metabolism is pyruvic acid (C in figure), and as a result of the catabolism of fatty acids

acetic acid is formed (G in diagram). It is now rather generally accepted that fatty acids arising from endogenous and exogenous fat are oxidized via a modified B-oxidation pathway as first described by Knoop[14]. According to this hypothesis, the terminal 2-carbon atoms of the fatty acid chain are successively removed as acetic acid.

That acetic acid or some other similar 2-carbon fragment or derivative is a product of fatty acid oxidation has been abundantly proved. Bloch and Rittenberg[15] by means of isotopically tagged fatty acids have provided excellent evidence for the formation of acetic acid, or some other 2-carbon fragment with the properties of acetic acid. Whether the 2-carbon fragment that arises from the oxidation of pyruvic acid is identical with the 2-carbon fragment resulting from fatty acid oxidation is as yet uncertain and currently the subject of intense investigation. This subject has recently been reviewed by a number of investigators[16].

Whatever the nature of the 2-carbon fragment, it is certain that acetic acid is rapidly swept into the tricarboxylic acid cycle and oxidized to carbon dioxide and water, and that the rate of this oxidation is markedly influenced by the rate of pyruvate production. In a recent experiment the author and his associates[17] found that when radioactive sodium acetate labeled in the carboxyl carbon is injected intraperitoneally into a normal rat, an amount greater than 90 per cent of the activity originally in the injected acetate was expired in the respiratory carbon dioxide. Approximately 80 per cent of the total activity was expired within the first two hours. Only 1.78 per cent of the activity was found in the fatty acids isolated from the tissues of the animal. The oxidation of acetic acid is normally, therefore, very rapid, and this may in some measure account for the frequent failure of investigators to detect, with ordinary chemical and physical techniques, acetic acid, or some close derivative, in animal tissues. These same investigators[18] have also noted that when non-isotopic pyruvate was administered along with the labeled acetate, the rate of oxidation of acetate was diminished and the incorporation of activity into the tissue fatty acids was enhanced. In the light of the findings just described, it is not surprising that any diminution in the production of pyruvate, as in starvation or diabetes mellitus, elicits a prompt and immediate response by increasing the rate of fatty acid oxidation, possibly for the purpose of remedying the loss of energy-forming reactions resulting from the impairment of carbohydrate metabolism. The probability that this sensitive, trigger-like mechanism is controlled by some as yet unknown endocrine control is recognized.

The Development of Ketosis

For a number of years, because of the observation that ketone bodies accumulate in the starving animal and in the experimental animal rendered diabetic, some doubt has been cast on the B-oxidation theory. Additional support for doubt was obtained through the well known clinical fact that ketosis invariably follows as a consequence of unregulated diabetes. Since under the conditions just indicated acetoacetic acid (a ketone body), and not acetic acid, appears to accumulate, the ketone bodies came to be regarded as primary oxidation products of the fatty acid chain.

Within recent years some revision of this concept has been necessary because work by Barnes and associates in the intact animal[19], and Weinhouse, Medes and Floyd in liver slices[20] has shown that ketone acids are formed as the result of condensation of two molecules of acetic acid. Weinhouse and his co-workers incubated liver slices with an 8-carbon fatty acid (octanoic acid) which had been previously labeled with isotopic carbon (C^{13}) in the carboxyl group. The ketone acid which was isolated from the mixture was found to contain the labeled carbon in both the carboxyl or terminal carbon and in the keto or carbonyl carbon. The activity was nearly equally distributed between the two carbon atoms. Acetoacetic acid with such a distribution of isotopic carbon could not have resulted from the splitting of the 8-carbon acid into two 4-carbon compounds. Rather, first there must have been a splitting of acetic acid from the larger fatty acid, followed by a condensation of two acetic acid molecules to form acetoacetic acid. It becomes unnecessary, therefore, to assume that acetoacetic acid lies on the main path of fatty acid oxidation, but rather that it is formed as a result of a side reaction (G and H in figure).

Liver tissue appears to have a limited capacity for oxidizing fatty acids beyond the acetic acid or 2-carbon stage. With a limited ability to oxidize the product, acetone bodies are formed which are dispatched to the extrahepatic tissues for complete oxida-

tion. A small but definite blood concentration of ketone bodies is therefore normal. In the diabetic animal, Chaikoff and Soskin[21], and also Barnes[22], have found that the tissues oxidize ketone bodies as readily as do those of normal animals. The ketonemia and resulting ketosis of the person with diabetes would not appear, therefore, to be the consequence of an impaired ability to oxidize and remove these bodies. Rather, the accumulation of ketone bodies in the diabetic person appears to be the resultant of a stimulated fat catabolism on the one hand, and on the other on a limit placed on the rate through which acetic acid can be swept into the tricarboxylic acid cycle and burned to carbon dioxide and water. In the former case the stimulation appears to be due to the need to compensate for the loss of energy-providing reactions because of the defect in carbohydrate metabolism. In the latter case the limit need not be an abnormal one, although this possibility is recognized in view of the stimulating effect of pyruvate on acetate oxidation previously described, but rather a physiologic limit to the number of acetic acid molecules, or 2-carbon fragments, that can be taken into the tricarboxylic acid cycle during a given period of time. The net result is an accumulation of acetone bodies (acetoacetic acid and B-hydroxybutyric acid) from the acetate formed. This condition would persist until the initial carbohydrate defect is corrected.

Relationship of Diabetes Mellitus to Hypercholesteremia

It is reasonably well established that ketosis of any appreciable duration resulting from a variety of causes leads to hypercholesteremia. The work of Bloch and Rittenberg[23] using labeled acetate has shown that the carbon atoms of acetic acid contribute to the molecular structure of both cholesterol and fatty acids. Barnes and associates[19], and Weinhouse and co-workers[20] have shown that the labeled carbon of acetic acid can be utilized in the synthesis of acetoacetic acid (ketone body), presumably by the condensation of two molecules of acetic acid. It is perfectly feasible, therefore, for the animal organism to use the carbon atoms of acetic acid, arising as the end product of B-oxidation of fatty acids, (1) for the resynthesis of fatty acids, presumably by a reversal of the catabolic pathway (G in the figure); (2) for the production of ketone bodies, apparently by a side reaction previously described (H in figure); and (3) for the synthesis of cholesterol by mechanisms as yet unknown.

Although the exact nature of the stimulus influencing the direction of synthesis is as yet unknown, a suggestion can be found in the liver tissue slice experiments reported by Bloch[24]. In these experiments Bloch found that when liver tissue slices were incubated with acetate, labeled in the carboxyl group with radioactive carbon (C^{14}), there was an increased incorporation of radioactive carbon in the fatty acids isolated from the mixture as the concentration of non-isotopic pyruvate added was increased, and a marked lowering of the isotopic concentration in the cholesterol isolated. Apparently, the direction of incorporation of carbon from acetate, whether toward fatty acids or cholesterol, is very sensitive to fluctuations in the concentration of pyruvate. In the presence of increasing concentrations of pyruvate, the emphasis would appear to be in the direction of tissue fatty acid synthesis, while in the presence of decreasing concentrations of pyruvate, there is a shift in emphasis to cholesterol synthesis.

On the basis of these experiments, it would appear reasonable to postulate that in conditions where very large quantities of fatty acids are being catabolized, as in diabetes, and where there is presumably a diminished production of pyruvic acid because of the impaired carbohydrate metabolism, greater quantities of acetic acid than normally occur would accumulate. Since it would appear that there are limitations to the rate at which acetic acid can be incorporated into and oxidized by the tricarboxylic acid cycle, the excess acid could be handled by the tissues for resynthesis of tissue fatty acids and the synthesis of cholesterol and ketone bodies. In the diabetic patient, if the results of the experiments by Bloch just described are correctly interpreted, where there is an impaired carbohydrate metabolism, the emphasis on the synthetic mechanism would be in the direction of cholesterol. It is a clinical fact that ketosis is associated with diabetes. Therefore, cholesterol and ketone bodies would tend to accumulate in the tissues. The hypercholesteremia of diabetes then would be as much a fundamental part of the biochemical de-arrangement of the diabetic state as ketosis.

Hypercholesteremia and Arteriosclerosis

The fact that the blood cholesterol tends to be high in uncontrolled and improperly controlled diabetes appears to be related to the arteriosclerosis and vascular degeneration resulting from the disease. Insulin and dietary control apparently have not solved this problem[25]. According to Thorn and Forsham[26], vascular lesions characterized by atheromatous changes are the most important changes secondary to long-standing diabetes, and are responsible for the death of more than 50 per cent of all patients who have had diabetes longer than 10 to 15 years. There is also evidence to indicate that vascular disease progresses more rapidly in poorly regulated diabetes. It is not known at present why diabetic patients show arterial changes, although it has been assumed that these changes are secondary to the disordered fat metabolism and hypercholesteremia. The subject of lipid metabolism and atherosclerosis has been reviewed recently by Gould[27].

At the present time, the relationship between an elevated serum cholesterol and the occurrence or progression of arteriosclerosis in man is still poorly understood. It has been repeatedly demonstrated that it is difficult to influence the serum concentration of cholesterol by feeding cholesterol. During the past several years a number of significant observations concerning the relationship of hypercholesteremia and atherosclerosis have been made. Buck and Rossiter[28] have analyzed chemically the lipid content of 31 aortas removed from males with ages distributed from birth to 85 years. The areas in the aorta showing atheroma had an increased concentration of total lipid, total cholesterol, total phospholipid, and neutral fat as contrasted with areas that appeared essentially normal. When 17 of the aortic sections were arranged according to the degree of atheroma, it was found that the cholesterol ester content diminished in proportion to the free cholesterol from a ratio of 1.4:1 to 0.4:1, from the least to the most severely affected sections.

Several papers have appeared recently that may throw some light on the knotty problem concerning the lack of correlation between hypercholesteremia and the development of the disease. Steiner and co-workers[29] have reported the successful production of atherosclerosis in dogs with depressed thyroid function by the feeding of thiouracil and high-cholesterol diets. This work is of interest in regard to the experimental approach to the problem, since it is believed that the diseases of the dog resemble those of man more closely than do diseases in the chicken or rabbit, other species which have been used extensively experimentally.

Messinger and associates[30] have reported that in human feeding experiments the feeding of egg yolk powder was followed by an increase in serum cholesterol, whereas feeding of cholesterol without egg yolk powder produced no change in serum levels. They interpret their results as being due to an as yet unidentified substance which alters the absorption or deposition of cholesterol. The agent does not appear to be lecithin, since Steiner and Domanski[31] have observed that serum cholesterol decreases when soybean lecithin is fed to human beings.

Recently in a series of experiments Gofman and his associates[32] have reported a high degree of correlation between proved (according to Gofman) coronary atherosclerosis and the presence of an abnormal lipoprotein, or "giant molecule," in blood serum. According to these workers, only a specific portion of the serum cholesterol is associated with the abnormal lipoprotein and the development of atherosclerosis. Furthermore, according to these authors, the presence of these cholesterol-bearing lipid and lipoprotein molecules cannot be predicted from the analytic serum cholesterol level. This hypothesis, if confirmed, would resolve the discouraging reports of the lack of correlation between high serum cholesterol levels and the development of the vascular lesions.

That all is not clean-cut, however, is indicated in a statistical study of existing data by Keys[33]. This author, contrary to Gofman and his group, finds that there is a substantial correlation between the concentrations in human blood serum of total cholesterol and the "giant" or abnormal lipoprotein molecules. Furthermore, the claim is made that there is no evidence to indicate that the abnormal lipoprotein is more critical than that of total serum cholesterol in predicting the development of atherosclerosis.

It is hoped that a resolution of these conflicting views and continued laboratory investigation along the lines indicated by Gofman will resolve to some degree the difference of opinion as to whether a dietetic re-

striction of cholesterol is of benefit for patients with atherosclerosis, particularly in view of the fact, previously described, that the cholesterol needs of the body can be supplied by biosynthesis independently of the diet.

Summary

An attempt has been made to show how continued laboratory research concerned with unraveling the intricate pattern of intermediary metabolism is of immense value in assisting in the understanding of the origin and progression of metabolic diseases.

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FIBRINOLYTIC ENZYME SYSTEM OF DOG SERUM*

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The phenomenon of whole blood clot lysis has been observed occasionally in blood taken from patients suffering from certain severe illnesses, for example: surgical shock[1], medical shock[1, 2], methyl alcohol poisoning[2b], and liver disease[2b, 3], and following sudden death[4]. This complete dissolution of the fibrin clot is caused by a proteolytic enzyme, fibrinolysin (lysin, plasmin, tryptase). The mechanism whereby this enzyme appears in the circulating blood has been poorly understood. There are a number of components

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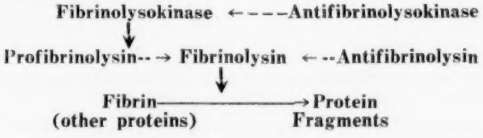
of the fibrinolytic enzyme system which we have undertaken to explore before attempting to produce the pathologic appearance of the active enzyme experimentally. Most of our studies have concerned dog serum and its fractions. Our conception of the components of this enzyme system in normal dog serum is:



Normal serum does not contain the enzyme in its active form—that is, as fibrinolysin (lysin)—but does contain the inactive precursor, profibrinolysin (prolysin). Our studies indicate that an activator, a fibrinolysokinase, is necessary for the conversion of profibrinolysin to fibrinolysin. Although fibrinolysokinase is present in serum, it is probably prevented from acting (under physiologic conditions) by the presence of an antifibrinolysokinase. This last substance, or group of substances, has not yet been isolated and studied, but we have obtained indirect evidence which suggests its existence. Antistreptokinase[5] is a known example of an antilysokinase. Antifibrinolysin[6], a normal serum protein fraction, acts to neutralize active fibrinolysin and other proteases (*e.g.* trypsin[6a]).

The studies presented in this paper concern the *in vitro* activation of profibrinolysin by fibrinolysokinases of various types. Fibrinolysokinase activity has been found (1) in certain bacterial products — for example, streptokinase[7] which activates human prolysin, and staphylokinase[8] which activates prolysins from a number of animals, (2) in tissues[9], and (3) in serum[10].

In most experiments we have used a purified dog serum profibrinolysin (see appendix), which is free from antifibrinolysin and serum fibrinolysokinase. Activation of this profibrinolysin is measured by assaying (see appendix) the amount of fibrinolysin which appears as this prolysin is converted to the active enzyme. In preliminary studies an incubation temperature of 37 C. was employed, as activation was rapid at this temperature. It was found, however, that fibrinolysin deteriorates rapidly at 37 C., and accurate estimations of the total amounts formed were impossible. To overcome this difficulty, incubation was carried out at 5 C., at which temperature the formed fibrinolysin was moderately stable over several weeks.

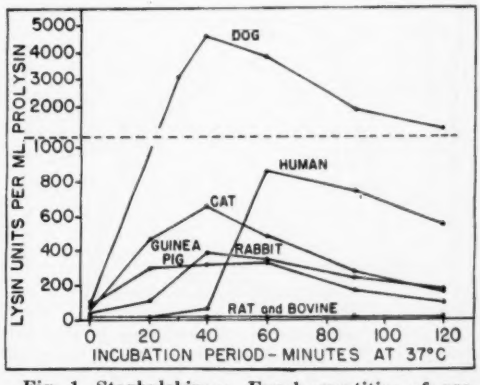


Fig. 1. Staphylokinase. Equal quantities of prolysins prepared from various animal sera were incubated with 2 per cent staphylokinase at 37 C. and the lysin content of each mixture titered at intervals. (From Lewis and Ferguson[8c]. Courtesy, the American Journal of Physiology.)

I. *Activation of Profibrinolysin by Staphylokinase*[8a]

Activation of human profibrinolysin by streptokinase was studied in some detail by Milstone[7a] and Christensen[7b]. The fact that certain strains of *Micrococcus aureus* also produced a fibrinolysokinase, staphylokinase, was demonstrated by Lack[8b] and Gerheim and others[8a]. In a survey of 40 bacterial types[11] we were unable to find any other bacterium producing fibrinolysokinase activity. Therefore we chose staphylokinase (see appendix) for intensive study.

Species reactivity

In figure 1 are presented the results of incubating prolysins prepared from sera obtained from seven animal species with equal quantities of 2 per cent staphylokinase at 37 C. Dog prolysin was quickly activated to yield a high titer of fibrinolysin. Human, cat, guinea pig, and rabbit prolysins showed some activation, but yielded considerably lower amounts of fibrinolysin. Rat and bovine prolysins were not activated by staphylokinase. These marked species differences could reflect: (1) incomplete, or lack of, activation by staphylokinase; (2) varying total serum profibrinolysin contents; or (3) varying percentages of the total serum profibrinolysin present in the isolated fractions.

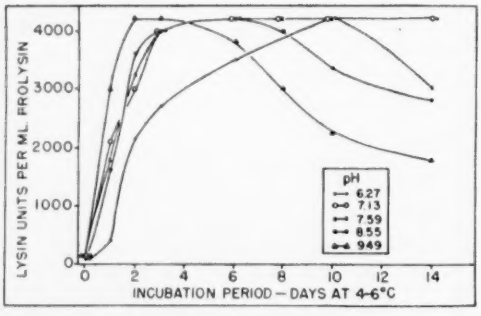


Fig. 2. Staphylokinase. Staphylokinase—dog prolysin mixtures were buffered at the specified pH levels (glass electrode) and incubated at 4-6 C. for 14 days. At intervals the mixtures were sampled, pH's adjusted to 7.7 ± 0.2 and lysin contents titered. (From Lewis and Ferguson[8c]. Courtesy, the American Journal of Physiology.)

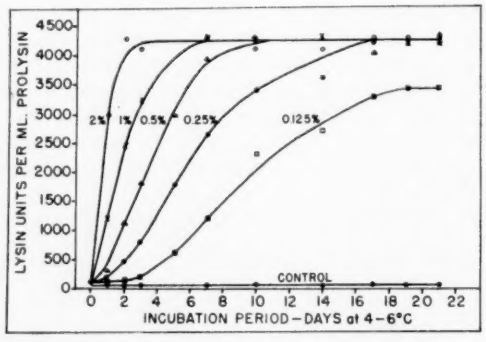


Fig. 3. Staphylokinase. A series of staphylokinase dilutions were incubated (pH 7) separately with the same quantities of dog prolysin and lysin contents determined at intervals. (From Lewis and Ferguson[8c]. Courtesy, the American Journal of Physiology.)

In the case of bovine profibrinolysin the first theory seems correct. No activation by staphylokinase has ever been observed during studies of whole bovine serum and many of its fractions.

Effects of pH

Dog prolysin was activated by 2 per cent staphylokinase in the presence of buffers of various *p*H levels at 5 C. As shown in figure 2, *p*H levels between 6.27 and 9.49 had no effect upon the total fibrinolysin yield. Staphylokinase activation was most rapid at alkaline *p*H and slowest at acid *p*H. Stability of formed fibrinolysin was greatest at about *p*H 7.0, and this *p*H level was chosen for other studies.

Kinetic studies

The same quantities of dog prolysin were activated by different amounts of staphylokinase at *p*H 7.0. As shown in figure 3 the fibrinolysin yields were the same with staphylokinase concentrations of 2 per cent to 0.25 per cent. Activation rate was more rapid with the more concentrated staphylokinase solutions. On the other hand, if the staphylokinase concentration was kept constant and the profibrinolysin concentration varied, as shown in figure 4, the fibrinolysin yield was directly proportional to the profibrinolysin concentration. These studies indicate that staphylokinase acts in an enzyme-like manner in the conversion of dog profibrinolysin to fibrinolysin.

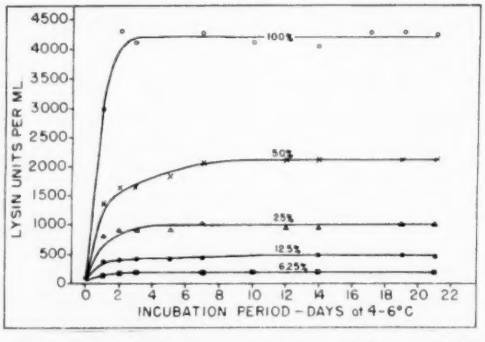


Fig. 4. Staphylokinase. A series of dog prolysin dilutions were incubated (pH 7.0) with the same quantities of 2 per cent staphylokinase and lysin contents determined. (From Lewis and Ferguson[8c]. Courtesy, the American Journal of Physiology.)

II. *Activation of Profibrinolysin by Tissue Fibrinolysokinase*

Astrup and Permin[9a] and Permin[9b] first reported that certain tissues contained kinase activity. Tagnon and Petermann[9c,d], in a study of mouse lung tissue, found the kinase activity associated with the "microsome" fraction as prepared by the differential fractionation method of Claude[12]. Data presented below include an extensive survey of dog tissues for kinase activity and the preparation of a purified lung fibrinolysokinase. Lung tissue was chosen for special study, as it proved a good source of fibrinolysokinase and could be readily freed from blood by perfusion of the fresh lung with saline.

Survey of various dog tissues for fibrinolysokinase activity[9c]

Thirty-five different dog tissues were obtained, thoroughly ground, and fractionated by sedimentation at different centrifugal forces into seven fractions. Each fraction from each tissue was studied for kinase activity by incubation with dog prolysin. When present, significant concentrations of kinase activity were usually found in the *first* fraction, sedimented at 1900 g, which consisted of gross particles, connective tissue, and large cellular fragments; the *fourth* fraction, sedimented at 2700 g, containing Claude's "large granules"; and the *sixth* fraction, sedimented at 42,000 g, consisting of Claude's "microsomes." Activity was consistently absent in the seventh fraction, which consisted of the material not sedimented by 42,000 g for 90 minutes. None of the tissue fractions showed fibrinolytic activity in the absence of dog prolysin. Tissues which contained significant concentrations of fibrinolysokinase were: lung, uterus, pancreas, gallbladder, ovary, lymph gland, placenta, urinary bladder, ureter, vein, bone-marrow, brain, spinal cord, and meninges.

Fibrinolysokinase in different species

"Microsome" fractions were prepared from the lungs of various animal species and tested for their ability to activate human, dog, and bovine prolysins. Mouse, cat, dog, and human lung fractions were able to activate dog and human prolysin to give moderate yields of fibrinolysin and bovine prolysin to give a somewhat lower yield. Rat and guinea pig lung fractions were somewhat less active on all three prolysins. Rabbit lung produced moderate activity from dog prolysin, but only a trace from human and bovine prolysins. Inexplicably, bovine lung fraction had no activating effects on any of the prolysins.

Purification of dog lung fibrinolysokinase

As most of the kinase activity of dog lung was found in the large granule and microsome fractions, these two fractions were combined as crude lung fibrinolysokinase (see appendix for preparation). This fraction probably contains a number of intracellular enzyme systems and many attempts were made to isolate the kinase in a more purified form. The final procedure adopted (see appendix) involves partial dispersement of the granules at low ionic strength and *p*H of 7.5 to 7.7. Part of the kinase then becomes soluble and can be recovered by acid precipitation of the supernatant after removal of the remaining "microsomes" by sedimentation at 42,000 g. Chemical studies of this purified lung fibrinolysokinase suggest that it is, or is closely associated with, a protein having an isoelectric point at *p*H 3.8.

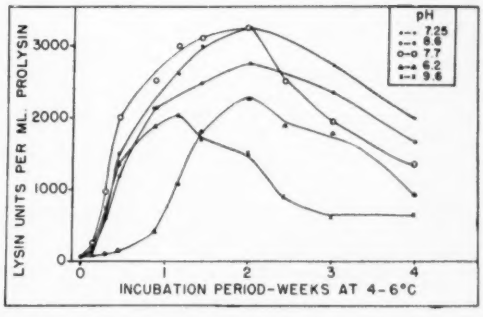


Fig. 5. Lung Fibrinolysokinase. Lung fibrinolysokinase—dog prolysin mixtures were buffered at the specified pH levels (glass electrode) and incubated. At intervals the mixtures were sampled, pH's adjusted to 7.7 ± 0.2 and lysin contents determined.

*Effects of p*H

The effects of *p*H on activation of dog prolysin by purified dog lung kinase were studied by incubating these substances together in the presence of buffers at various *p*H levels. As shown in figure 5, *p*H had a marked effect, not only on the rate of fibrinolysin formation, but also upon the yields of fibrinolysin. Highest yields were obtained at *p*H 7.25 and 7.7. Even these "maximal" yields of 3200 units were not as great as the potential yield of 4600 units obtainable from this lot of profibrinolysin by staphylokinase activation.

Reaction kinetics

Prolysin was incubated with various dilutions of purified lung kinase at *p*H 7.3. As shown in figure 6, both the rate of activation and the final fibrinolysin yields were dependent upon the concentrations of the kinase. In the next experiment the lung kinase concentration was maintained constant and the prolysin concentration varied. As shown in figure 7, the fibrinolysin yield was apparently dependent upon the initial prolysin concentration.

Simultaneous studies were made with staphylokinase on the prolysin employed in these experiments, and showed that the po-

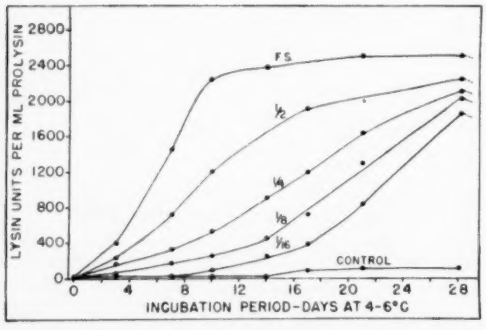


Fig. 6. Lung Fibrinolysokinase. A series of purified dog lung kinase dilutions were incubated (pH 7.3) separately with equal quantities of dog prolysin and lysin contents determined at intervals.

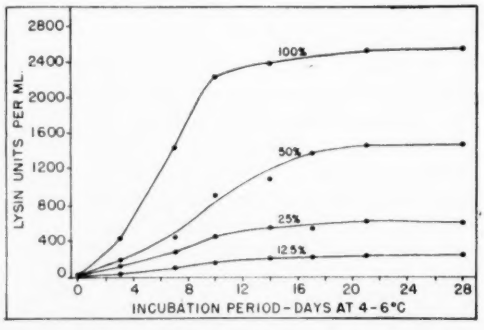


Fig. 7. Lung Fibrinolysokinase. A series of dog prolysin dilutions were incubated (pH 7.3) with full strength dog lung kinase and lysin contents titered at intervals.

tential fibrinolysin yield was 4200 units rather than the 2500 units obtained with the lung kinase. Addition of staphylokinase to the lung kinase — prolysin mixtures after four weeks' incubation did not increase the fibrinolysin yield, suggesting that no profibrinolysin remained in these mixtures. Deterioration of the formed fibrinolysin was not noted in these experiments. A possible explanation for the discrepancy in fibrinolysin yields between staphylokinase and lung kinase activation is that lung kinase converts prolysin to active lysin plus an inert protein, while staphylokinase converts it completely to lysin. Further investigation will be necessary to decide this point.

III. *Activation of Prolysin by Serum Fibrinolysokinase*

The spontaneous appearance of fibrinolysin in certain serum fractions[13,14] and in whole serum treated with chloroform[15,16] has been attributed to various mechanisms involving either (a) the removal of antifibrinolysin from a fibrinolysin-antifibrinolysin complex[15,17] or (b) an autocatalytic activation of profibrinolysin after removal of antifibrinolysin[18]. We have discarded both of these theories on the grounds that (a) after the removal of antifibrinolysin, fibrinolysin does not suddenly appear, but develops gradually; and (b) our dog profibrinolysin does not usually activate spontaneously, nor can it be activated consistently by the addition of fibrinolysin. Although fibrinolysin appears in whole serum after treatment with chloroform, it does *not* appear in the isolated profibrinolysin, which has been similarly treated. Therefore we have postulated that the so-called "spontaneous" activation of whole serum profibrinolysin is due to the action of a *fibrinolysokinase*—present in serum and independent of blood cellular elements. The experimental data presented below substantiate this theory.

Preparation and action of serum fibrinolysokinase[10]

Many attempts were made to isolate from serum a fraction which was free from profibrinolysin and antifibrinolysin, and which was capable of activating profibrinolysin. The serum fraction used in this experiment, Fraction K (see appendix), was prepared by ethanol fractionation. It was found to be free from profibrinolysin, but contained a trace of antifibrinolysin. Figure 8 illustrates the effect of this Fraction K on prolysin. After an initial lag the prolysin *plus* fraction K mixture (P+K) developed a high titer of fibrinolysin (3730 units) comparable to that developed by this prolysin plus staphylokinase (4200 units). The control curves (P, K) show that significant titers of fibrinolysin were not developed in the prolysin or the fraction K when they were incubated separately.

Are cellular elements responsible for serum fibrinolysokinase?

Although previous studies in this laboratory[11] did not show any appreciable fibrinolysokinase activity in erythrocytes, leukocytes or platelets, it was thought possible, especially in view of the work of Permin[9b], that blood cellular elements might be con-

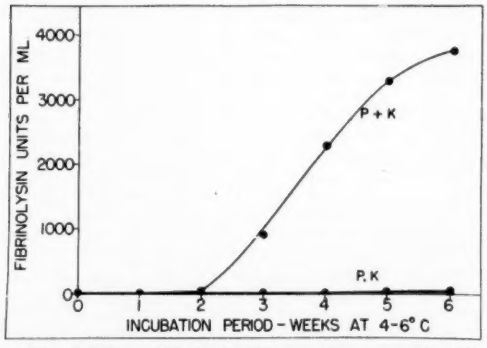


Fig. 8. Serum Fibrinolysokinase. Dog prolysin (P) and dog serum kinase (K) were incubated together (pH 7.3) and the lysin content determined (curve P + K). Controls (P,K) consisting of dog prolysin plus saline and lung kinase plus saline were also incubated and tested for lysin contents. (From Lewis and Ferguson[10]. Courtesy, the Society for Experimental Biology and Medicine.)

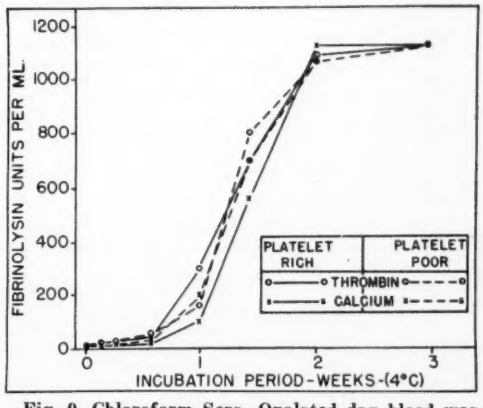


Fig. 9. Chloroform Sera. Oxalated dog blood was centrifuged at high and low speeds. The plasmas were recalcified or thrombinized and the resulting sera shaken with ½ volume of chloroform. Lysin contents of each serum were titered at intervals. (From Lewis and Ferguson[10]. Courtesy, the Society for Experimental Biology and Medicine.)

tributing fibrinolysokinase activity to serum. This problem was approached by following the appearance of fibrinolysin in chloroformed serum obtained from plasmas centrifuged at very low and very high speeds. The "platelet-rich" plasma was centrifuged at 320 *g* for 15 minutes and contained many erythrocytes, leukocytes, and platelets. The "platelet-poor" plasma was centrifuged at 42,000 *g* for 60 minutes and showed no cellular elements on smear. Sera were obtained from both plasmas in two ways: (1) by recalcification: IA, IIA, and (2) by thrombinization: IB, IIB. All sera were treated with chloroform in the same manner. As shown in figure 9, fibrinolytic activity developed at approximately the same rate and to the same extent in each serum, and we concluded that the blood cellular elements did not contribute to the fibrinolysokinase activity of serum.

Summary

Dog serum profibrinolysin can be activated to fibrinolysin by at least three fibrinolysokinases. Staphylokinase apparently acts as an enzyme in causing the complete conversion of profibrinolysin to the active enzyme. Although it acts over a wide *p*H range, the rate of activation is more rapid on the alkaline side. Fibrinolysokinase is present in a wide variety of tissues, of which lung has proved a potent and convenient source. Studies on a purified lung kinase show that it activates dog prolysin incompletely, apparently converting some to an inert protein rather than to the active enzyme. The *p*H range for maximal lung kinase activity lies between 7.25 and 7.7. Fibrinolysokinase is also present in serum, and is shown to be independent of the cellular elements. The kinase activity can be isolated in a serum protein fraction. Additional studies are underway to determine the mode of action of serum fibrinolysokinase.

Appendix

Assay of Fibrinolysin. Fibrinolytic activity is assayed by the rate of lysis of a standard fibrin clot, prepared as follows:

Tube 1: 0.5 ml. 1% Bovine Fraction I (Armour)*
0.5 ml. borate buffer, **pH** 7.7
(11.25 Gm. H_3BO_3, 2.25 Gm. NaCl, 4.00 Gm. $Na_2B_4O_7.10\ H_2O$ in distilled water to 1 liter)

Tube 2: 1.0 ml. test material
0.2 ml. thrombin (20 units/ml.)

Tubes 1 and 2 are mixed by pouring back and forth four times and the final tube placed in a 37 C. water bath. Lysis time is measured on a stopwatch from time of mixing to time of complete dissolution of the gelatinous fibrin clot, and is then converted to units by reference to a standard curve (fig. 10), prepared by determining lysis times of precise dilutions of human, dog, and bovine fibrinolysins. An arbitrary reference point of 100 units equal to a lysis time of five minutes was chosen. The **pH** optimum for fibrinolysis was found to be 7.7 to 8.25. All test solutions are adjusted to this range before assaying for fibrinolysin.

Preparation of Dog Serum Profibrinolysin (Prolysin). Hemoglobin-free oxalated plasma is recalcified and the resulting serum chilled to 0 C. and

*The final fibrinogen (clottable protein) concentration in the standard fibrin clot is 0.136 per cent. Each new lot of Fraction I is analyzed and the concentration adjusted so that 0.5 ml. contains 3.0 mg. of clottable protein.

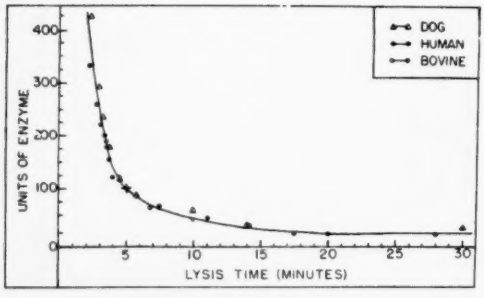


Fig. 10. Standard Fibrinolysin Curve. (From Lewis and Ferguson[6d]. Courtesy, the Journal of Clinical Investigation.)

brought to one-third saturation with ammonium sulfate (saturated solution at 0 C.). The precipitate, recovered by centrifugation, is dissolved in distilled water and dialysed 18 hours against cold running tap water (4 C.). The final precipitate is dissolved in one fourth the original serum volume (corrected for diluents).

Preparation of staphylokinase. Stock medium is prepared by dissolving 25 Gm. Bacto-Heart Infusion Broth, dehydrated (Difco) and 0.5 Gm. dextrose in 1 liter of distilled water and adjusting **pH** to 7.6. Small flasks of this medium are inoculated, incubated 20 to 22 hours at 37 C. and smears checked for purity of culture. The contents of these flasks are then poured into 1 liter flasks of stock medium, incubated 18 hours and smears again checked for purity. Bacteria are removed by centrifugation, the supernatant chilled to 5 C., and three times the volume of cold 95 per cent ethanol added. After standing 1 hour at 5 C. the resulting precipitate is recovered by centrifugation, suspended in absolute alcohol, and dried on a Büchner funnel by alternate washings with alcohol and ether. The dry powder is stored in a dessicator.

The best yields of active staphylokinase are usually obtained from freshly isolated strains of **Micrococcus aureus.** Survey of a group of strains may be necessary to choose a potent staphylokinase producer. This is done by inoculating 5 ml. tubes of stock medium with each strain, incubating 20 hours, removing bacteria by centrifugation and testing the supernatant by incubating it with prolysin for one hour at 37 C. and determining the fibrinolytic activity. Only those strains which produce lysis of the standard clot in three minutes or less are used to prepare the concentrated kinase.

Crude Lung Fibrinolysokinase. Freshly obtained dog lungs are perfused free from blood with 0.85 per cent sodium chloride (saline) and macerated in a Waring Blendor for five minutes with frozen saline, buffered at **pH** 7.3 to a final phosphate concentration of 0.0025 M (buffered saline). The mixture is strained, ground thoroughly in a mortar and centrifuged at 5000 RPM (4200 g) for 15 minutes. The sediment is again treated with the buffered saline ice in the Blendor and recentrifuged. The two supernatants are combined and centrifuged for 90 minutes at 16,000 RPM (42,000 g). The small grey sediment so obtained is washed with buffered saline and recentrifuged as before. This washed sediment is dispersed in a volume of buffered saline equal in milliliters to one-half the original lung weight in grams.

Purified Lung Fibrinolysokinase. Crude lung kinase is diluted tenfold with 0.0001 N NaOH and dialysed at 5 C. for 18 hours against repeated changes of 0.0001 N NaOH. The dialysed material is centrifuged at 42,000 g for 90 minutes. Although the sediment retains some kinase activity it is not used routinely. The cold, slightly opaque supernatant is brought to **pH** 3.8 by addition of 0.1 N HCl. A flocculant precipitate promptly forms and is collected by centrifugation (4200 g for 15 minutes). This precipitate is dispersed in saline and the **pH** adjusted to approximately 8.0, at which point most of the precipitate dissolves. Reprecipitation is carried out one or more times. The final precipitate is dispersed in a volume of saline equal in milliliters to one-fifth the original lung weight and the **pH** adjusted or buffered as desired.

Serum Fibrinolysokinase (Fraction K). Pooled whole dog serum is chilled to 5 C. and an equal volume of cold absolute ethyl alcohol added. The precipitate so formed is discarded, and an additional volume of alcohol equivalent to 24 per cent of the original serum volume is added. The resulting precipitate is collected by centrifugation and dissolved in one-third the serum volume of saline. Traces of alcohol are removed by passing a stream of air over the solution. The **pH** is adjusted to 7.3.

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VERATRUM

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CHAPEL HILL

Sometimes in the history of a useful drug there is a period during which it may be almost completely forgotten. Between the years 1918 and 1935 medical literature records practically no evidence of interest in the pharmacology of veratrum as a hypotensive agent except in the observations published in 1925 by William deBerniere MacNider[1]. At that time veratrum was considered by all but the oldest practicing physicians to be too dangerous to use. They had used the drug with good results in the treatment of eclampsia, where it slowed the heart rate and prevented convulsions. MacNider pointed out in his report that digitalis had passed through comparable periods of disfavor before sufficient pharmacologic study and subsequent standardization proved it to be a dependable therapeutic agent. He thought veratrum deserved the same careful study.

Using the crude tinctures of veratrum which were available then, MacNider demonstrated in dogs that the drug lowered blood pressure even when the bradycardia was prevented by atropine or vagus section. He thought that veratrum might be useful in the treatment of hypertensive crises. In the last few years pharmacologists and clinicians, working closely with the pharmaceutical chemists who have isolated active fractions and pure alkaloid compounds from *V. viride* and *V. album*, again raise the hope of safe and useful application of these substances in the management of the hypertensive patient.

Disputes over the relative merits and dangers of veratrum are nearly as old as the beginnings of medicine itself. Ctesias (*ca.* 461 B.C.) wrote in his book on veratrum that he regarded the drug as a dangerous one; this was just one of the many points on which he disagreed with Hippocrates. There is repeated evidence in the writings of the ancients that veratrum was used by the medical men of Greece, Assyria, and Egypt. The American Indians, among them the Apaches, Penobscots, and Micmacs, employed veratrum in their medical practices.

Definition

What is veratrum? Veratrum is the generic name for two plants of the order Liliaceae, namely *Veratrum album*, which grows in the Old World, and *Veratrum viride*, which is indigenous to North America. These plants were for many years misnamed "hellebore." The term "veratrum" is also used to describe the alkaloid extracts derived from these plants, the rhizomes being the usual source of the extracts. Veratrine, on the other hand, is the name applied to extracts of the sabadilla plant, which grows in Mexico, these extracts being made from the seeds[2]. Some of the alkaloids extracted from sabadilla resemble chemically and pharmacologically certain alkaloids from *V. album* and *V. viride*.

Isolation and Purification of Veratrum Alkaloids

The chief objection to veratrum for many years was the fact that the preparations which were available were not dependable. Their potency varied widely from one lot to another, and preparations made by different manufacturers were not comparable. The fluid extract and the tincture, both prepared from the rhizome of the Veratrum plants, were among the earlier preparations. It was not until recently that it was recognized that the alkaloids derived from *V. album* differed from those from *V. viride*. The first purified alkaloids were described in 1820, but up to 15 years ago at least half of the alkaloidal content of veratrum was still in the amorphous fraction. In the last few years several active alkaloids have been crystallized from this fraction, but there are probably many more yet to be isolated.

One can readily imagine some of the difficulties in studying the veratrum alkaloids

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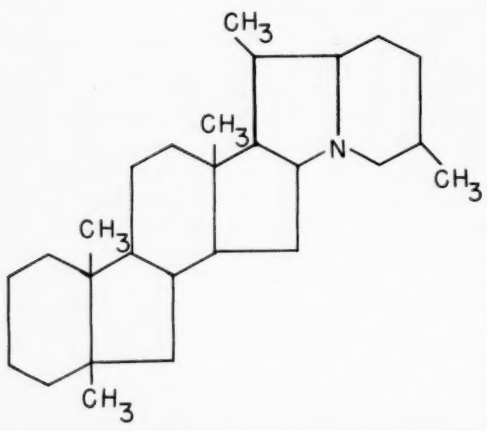

Fig. 1. Nucleus of a typical veratrum alkaloid.

when he considers that already more than 20 different alkaloids have been isolated and purified. In nature, veratrum alkaloids occur as alkamines, esters, and glycosides, besides the as yet non-purified amorphous fractions.

The nucleus of the purified crystalline alkaloids exhibits a striking similarity to that of the heterocyclic alkaloids of Digitalis, Aconite, Solanum, and Zygadenus. There is a marked similarity to the smaller nuclei of the steroid hormones. This similarity has prompted pharmaceutical chemists to attempt the synthesis of cortisone from jervine, one of the most abundant of the isolated veratrum alkaloids, which has little if any potency as a hypotensive drug[3].

There are at least three proprietary preparations on the market. Veratrone (Parke, Davis and Company) is an aqueous extract of the rhizome of *V. viride*. It is assayed chemically for total alkaloids. Vertavis (Irwin, Neisler and Company) is a tablet prepared from the whole rhizome. It is assayed using the production of cardiac arrest in the water flea *Daphnia magna* as an estimate of its potency. Veriloid (Riker Laboratories) is a tablet containing a constant fraction of the total alkaloids. It is assayed for hypotensive potency in the anesthetized dog.

Mode of Action

The pharmacology of veratrum alkaloids is almost unbelievably complex. The bradycardia induced by veratrum has been proven to be reflex in nature, with both afferent and efferent fibers running in the vagus nerves. The vasodilation and lowering of blood pressure following administration of veratrum has been thought variously to be a direct action on arterioles, a reflex action dependent upon special receptors in the heart, lungs, and carotid sinus area, or the result of direct action on the vasomotor center of the central nervous system.

Recent investigation has ruled out the possibility of direct action on the arterioles[4], and the special receptors in the heart, lungs, and carotid sinus area have been shown to be related principally to the bradycardia, but are not necessary to the hypotensive effect[5]. Ingenious experiments devised to separate the circulation in the head of a dog from the circulation in the rest of its body suggest that the receptors responsible for initiating the vasodepression are located in the head[6]. It has been established that veratrum does not block the effects of stimulation of sympathetic nerves, but on the other hand veratrum vasodilatation does not occur in sympathectomized limbs. These recent observations suggest that veratrum may act on a center or centers in the central nervous system to diminish sympathetic discharges which normally travel via sympathetic nerves to produce constrictor tone of arterioles. Another possibility is that veratrum may operate via vasodilator nerves which accompany these sympathetic nerves[7].

Certain of the veratrum alkaloids have been shown experimentally to increase the force of contraction of the heart. Continued administration of certain proprietary veratrum preparations has been seen to result in a decrease in heart size and a reversion of electrocardiographic findings toward normal in hypertensive patients[8].

After the blood pressure is reduced in patients who are given veratrum, blood flow through renal, hepato-portal, and skeletal muscle circuits is maintained at normal rates. Glomerular filtration rate and renal plasma flow are normal at the lowered level of blood pressure. Cardiac output is unchanged in the normal heart, but in the failing heart cardiac output may increase with a reduction in the pressure in the pulmonary circuit[7, 9]. Clinical observers have reported that they have seen retinal arterioles dilate in patients who were given veratrum preparations.

Avoidance of Undesirable Side Effects

Two objectionable effects of early vera-

trum preparations were an emetic action and a depression of respiration. It has since been shown that the dose which produces respiratory depression is much higher than that which is necessary to lower blood pressure. The emetic action of veratrum is produced in two ways, through local irritation of gastrointestinal mucosa and by direct action on the vomiting center. Parenteral administration circumvents the local effect, and adjustment of dosage of both oral and parenteral preparations will often avoid nausea and vomiting of central origin. Veriloid is reported to cause less nausea and vomiting than did earlier preparations[10].

Clinical Applications

In eclampsia

One of the most encouraging applications of veratrum in therapeutics is in the treatment of eclampsia. Older practitioners in the South who had used it observed that if the heart rate were reduced and maintained at 65 per minute or below, the eclamptic patient would be free from convulsions. It appears now that the hypotensive effect which accompanied the bradycardia was more important. The bradycardia served as a rough guide to the control of dosage in the cruder preparations which were then available.

More recently, in a long series of cases of frank eclampsia where veratrum was administered after the first convulsion, only 2 maternal deaths occurred in 120 consecutive cases[11]. These 2 deaths were ascribed to sepsis and not eclampsia. Besides veratrum, the patients received magnesium sulfate orally and intramuscularly, forced fluids, alkalies, a low protein diet, minimal sedation, with coservative management of pregnancy and delivery. Veratrone, the aqueous extract of veratrum, was used in these patients.

An interesting difference in the response to veratrum of the normal pregnant patient and the toxemic patient has been reported[12]. A certain dose of veratrum lowered the blood pressure of patients with toxemic pregnancy, but not those with normal pregnancy. In contrast, a given dose of tetraethyl ammonium chloride lowered the blood pressure in normal pregnancy but not in toxemic pregnancy.

In hypertension

Clinical trials of the available veratrum preparations in hypertensive patients have resulted in varying degrees of success[8,10,13,14,15]. Some investigators recommend their routine use in hypertension. Others feel that they should be reserved for use in hypertensive crises. Still others suggest that veratrum should be used in hypertension resistant to other forms of treatment, and in hypertension accompanied by cardiac failure.

One group has been studying the clinical action of protoveratrine, a purified ester alkaloid from *V. album*. These workers have administered this single alkaloid to hypertensive patients and demonstrated that it has high hypotensive potency in clinical doses without emetic activity[16]. This particular alkaloid, which is administered parenterally, is not available commercially. Two other pure alkaloids, germitrine and germidine, recently isolated from the amorphous fraction of V. *viride*, have been shown to have high hypotensive potency in man, but they appear to have more emetic effect[17].

Conclusion

There are still many things we would like to know about how veratrum works. But recent research has given us some understanding of its mechanism of action. The studies on different fractions of the crude preparations raise the hope that out of this mixture of alkaloids there will be obtained an agent which will promote depression of the blood pressure in hypertensive patients without undesirable side effects.

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THE SECRETION AND ENTEROHEPATIC CIRCULATION OF BILE ACIDS: REPLACEMENT OF BILE ACIDS IN BILIARY INSUFFICIENCY

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An inadequate flow of bile into the intestinal tract can result from a number of conditions, such as severe liver disease, biliary fistulas, biliary obstruction by gallstones or tumors, and congenital atresia of the bile ducts. In severe hepatic disease, the bile which is secreted may be deficient in bile acids. If deprivation of bile persists for several weeks or months in experimental animals or in human beings, nutritional and digestive disturbances, anemia, and a tendency toward abnormal bleeding may ensue.

Biliary insufficiency often causes inadequate intestinal absorption of fat-soluble vitamins, which can result in serious manifestations of avitaminosis even when normal amounts of the vitamins are included in the diet.[1]. The relationship of biliary insufficiency to symptoms such as anorexia, nausea, and distention which accompany certain diseases of the liver and of the gastrointestinal tract has not been determined in detail, but such relationships have been suggested[2]. The bile acids are the constituents of bile which are principally responsible for its effectiveness in promoting the intestinal absorption of fats and fat-soluble vitamins[1], and the bile acids are considered to be concerned in the relief of other symptoms of biliary insufficiency by replacement therapy with preparations of desiccated bile[2a]. A brief review of the subject of the secretion of bile acids may be desirable at this time when the principal gross aspects of biliary secretion have been established, and when investigations of the details of the physiologic synthesis of bile acids are being commenced.

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Comparison of Human and Animal Bile

A comparison of the bile acid composition of the bile of man with that of the ox and of the hog is desirable, inasmuch as desiccated bile of the two latter animals often is used for replacement-therapy in man[2a, 3]. The bile acids of man, the ox, and the hog consist of a steroid nucleus, the aliphatic side-chain of which possesses a carboxyl group which usually is conjugated through amide linkage with glycine or taurine. In the bile of man and of the ox, approximately 50 to 75 per cent of the total quantity of bile acids* is conjugated with glycine, and the principal portion of the remainder is conjugated with taurine[4]. However, this proportion is somewhat variable, and often a considerable fraction of the bile acids in bile is in the "free" or unconjugated form[5]. The bile acids of hog bile are conjugated solely with glycine[4, 6].

The bile acids of these three animals differ also in the substituents of the steroid nucleus and in the relative proportions in which they are present in the bile. The principal bile acid (steroid portion of the conjugated acid) of hog bile is a-hyodesoxycholic acid (3,6—dihydroxycholanic acid)[6]. The principal bile acid in the bile of man and of the ox is cholic acid (3,7,12—trihydroxycholanic acid), but important amounts of desoxycholic acid (3,12—dihydroxycholanic acid) and chenodesoxycholic acid** (3,7—dihydroxycholanic acid) also are present[7]. Hyodesoxycholic acid has not been found in the bile of man and the ox, and cholic acid is absent from the bile of the hog. Traces of lithocholic acid (3-hydroxycholanic acid) have been found in ox bile[8], in the bile of man[9], and in a gallstone from the gallbladder of a hog[10]. Appreciable quantities of keto-bile acids have been found in hog bile (principally 3-hydroxy-6-ketocholanic acid[11]) and in the

*It is customary to refer to the bile "acids" in bile, although it is understood that at the *p*H of bile these acids are almost completey ionized as anions, which are matched by sodium, potassium, and calcium cations.

**This bile acid is also termed anthropodesoxycholic acid.

Table 1

Composition of an Average Sample of Gallbladder Bile of the Hog

Cation and Anion Content in milli-equivalents per liter

Na	K	Ca	Mg	Free Choline	Total cations
175	19.7	9.8	4.8	0.76	210.1
Cl	HCO_3	Bile Salt			Total anions
32.0	15.7	165			212.7

Bile acids (Gm. per 100 ml. of bile)

Hyodesoxycholic acid	3-Hydroxy-6-ketoallocholanic acid	Total bile acids	Glyco conjugated	Total conjugated
5.69	0.77	6.46	5.49	5.91

Miscellaneous Organic Constituents (mg. per 100 ml. of bile)

Cholesterol	Bilirubin	Mucin	Total Fatty Acids after Hydrolysis	Total Phospholipid
161	36.5	692	1470	1680

bile of the dog[12]. It is not certain whether these keto bile acids are produced in the natural biliary secretory processes of these animals or whether they are formed by bacterial oxidation of the hydroxycholanic acids in the intestinal tract, from which they might be reabsorbed into the enterohepatic circulation. Such bacterial oxidation of hydroxycholanic acids occurs in the cecum of the guinea pig[13].

Comparison of hog bile before and after desiccation

Comparative analyses have been reported for pooled samples of fresh gallbladder bile of the hog before and after desiccation by a vacuum process[11b]. Hog bile dried according to this method is soluble in distilled water to the extent of 13.9 Gm. per 100 ml. at 25 C. Nine grams of the dried bile corresponds to 100 ml. of the average sample of gallbladder bile of the hog. Solutions prepared from the desiccated bile are identical with the original fresh bile, with the exception of partial hydrolysis of phospholipids and of small increase in *p*H due to loss of carbon dioxide during drying. These preparations of dried bile are useful for replacement therapy[2a,3a]. Typical analyses of gallbladder bile of the hog are presented in table 1. Average concentrations of bile acids in ox gallbladder bile are recorded in table 2.

Table 2

Average Concentrations of Bile Acids in Gallbladder Bile of the Ox

Bile acid	Concentration (Gm. per 100 ml.)
Cholic acid	4.6
Total dihydroxycholanic acids	1.5
Desoxycholic acid	1.1
Chenodesoxycholic acid	0.4
Total bile acids	6.3
Total conjugated bile acids	6.1
Tauro-conjugated bile acids	3.1
Glyco-conjugated bile acids	3.0

The Formation of Bile Acids

It seems to be generally accepted that the liver is the principal if not the only site of formation of the bile acids[14a]. The recent work of Bloch and others[14b] has provided the first clear indication of a precursor in the physiologic synthesis of bile acids. Cholesterol labeled with deuterium in the rings and in the side-chain was administered intravenously to a dog in which an anastomosis had been established between the gallbladder and the pelvis of the kidney. The cholic acid isolated from the urine of this dog had an isotope concentration of the same order of magnitude as the cholesterol of the blood and of the bile. Assuming that the circulating cholesterol provided the immediate precursor, it was calculated that a minimum of two thirds of the cholic acid was formed from this sterol[14b].

Bloch and Rittenberg had demonstrated previously that acetic acid is one of the precursors of cholesterol in mice and rats[15], and presumably in other animals. They have shown that the liver is a site for the synthesis of cholesterol from acetate, whereas under comparable conditions no synthesis of this sterol was observed in kidney, testes, spleen, or the gastrointestinal tract[16]. It is not yet known whether cholesterol is an obligatory precursor for the physiologic synthesis of bile acids. There appears to be a possibility that at least a small fraction of the total bile acids might be synthesized directly from simpler precursors such as acetic acid. There also is no information available concerning the physiologic synthesis of the mono and dihydroxycholanic acids, or regarding the possible metabolic relationship of these bile acids with the trihydroxycholanic acid, cholic acid.

There is evidence that conjugation of the bile acids with glycine or taurine occurs after the complete synthesis of the steroid portion

of the molecule[17]. Josephson and others[17a] injected 250 mg. of cholic acid intravenously into cats and rabbits with biliary fistulas. During the first 30 minute period of rapid biliary excretion of the bile acid, the principal portion was excreted in unconjugated form, but in subsequent periods a greater and greater proportion of the total "extra" bile acids appeared in conjugated form. Whipple and others[17b,c] have concluded from experiments on the oral administration of cholic acid to dogs that conjugation occurs to the degree in which glycine or taurine is available for this process. These results are similar to those obtained by Griffith and Lewis[18] in studies on the conjugation of benzoic acid with glycine. Various investigators[19] have reported that cholic acid synthesis appears to depend upon the amount of protein in the diet. Smith and Whipple[19a] found that meat and meat products, including beef heart, kidney and liver, when fed to bile fistula dogs cause a notable rise in the output of bile acids.

It is not clear what relationship these earlier observations have to the more recent discovery[14b] that cholesterol is an important precursor of cholic acid. The stimulatory effects of ingestion of liver and kidney probably are not due to their content of cholesterol, since the feeding of cholesterol to animals with biliary fistulas does not cause an increase in output of cholic acid[19a]. This observation seems to be in conflict with the conclusion of Bloch and colleagues[14b] until one recalls that there always are large stores of cholesterol in the tissues of animals, and the *supply* of cholesterol for synthesis of cholic acid may not be a limiting factor. Perhaps the stimulatory effect of proteins may be a function of the intact protein, possibly mediated by a hormonal mechanism.

In this connection it may be pertinent to consider the report of Annegers and Friend[20] that the biliary output in dogs increases linearly as the casein content of the diet is increased, but the effect cannot be attributed to amino acids since hydrolyzed casein does not produce the same response. If the stimulation of cholic acid output involves a hormonal mechanism, it seems probable that the hormone is not secretin, inasmuch as Grossman and others[21] have found that secretin causes an increase in the volume-rate of flow of bile in human subjects with T tubes in the common bile duct, but the output of cholic acid is not increased. They concluded that secretin is a hydrocholeretic agent with an effect similar to that produced by injections of dehydrocholic acids[22a].

The Enterohepatic Circulation of Bile Acids

The existence of an enterohepatic circulation of bile acids has been recognized for many years, and the evidence has been summarized in reviews[4,22]. Such circulation is important, since it permits the accumulation of bile acids in the circuit to levels which would be unattainable by the normal rate of synthesis of bile acids if unaided by absorption from the intestine and recirculation through the liver and the biliary system.

Whipple and Smith[19a,23] have concluded that the rate of synthesis and the final quantitative level of bile acids in the circuit are dependent in part upon the diet of the animal. Berman and others[19b] explained the attainment of a relatively constant concentration of bile acids in the circuit on the basis of a steady-state equilibrium between synthesis and loss of bile acids. Irvin and Johnston have investigated some of the quantitative details of enterohepatic circulation in hogs with biliary fistulas[24] and in human patients with choledochostomy drainage[25]. Inasmuch as cholic acid and the conjugated cholates are absent from the normal biliary secretion of the hog, these bile acids were used as "tracers" with which various quantitative aspects of enterohepatic circulation could be evaluated[24]. The loss of conjugated cholates during an average circulation time of 10 hours was 9 to 11 per cent of the amount administered, or of the total amount in circulation if repeated doses had been given with intervening periods of recirculation. The percentage loss of hyodesoxycholic acid (the naturally occurring bile acid of the hog) during circulation was comparable to the loss of cholic acid. Thus cholic acid is efficiently handled by the hog, although it is not a naturally occurring bile acid in the biliary secretion of this animal.

The utilization of a "foreign" bile acid is of interest in connection with the use of animal bile for replacement-therapy in humans[2a,3a,25]. The average rate of synthesis of hyodesoxycholic acid in hogs was found to be 5.4 mg. per kilogram of body weight per hour, and the average equilibrium level of hyodesoxycholic acid attained by contin-

Table 3

Excretion of Various Bile Acids in the Choledochostomy Drainage Bile of Patient (Case 1), and the Effect of Oral Administration of Desiccated Hog Bile

Days Postoperative	Hyodesoxycholic Acid Content of Hog Bile Administered	Average Volume of Bile Excreted per Day	Concentration of Bile Acids in Drainage Bile				
			Cholic Acid	Desoxycholic Acid	Hyodesoxycholic Acid	Total Dihydroxycholanic Acids	Total Bile Acids
	(millimoles per day)	(ml.)	(millimoles per liter)				
2	0	425	0	0		0	0
3-4	0	370	0	trace		trace	trace
5-6	0	460	0	0.10		0.10	0.10
7-8	0	540	trace	0.15		0.20	0.20
9-10	0	380	0.30	0.20		0.32	0.62
11	0	510	0.60	0.40		0.63	1.23
12	0	580	1.2	0.46		0.82	2.02
13	0	540	2.9	0.70		1.20	4.1
14	0	530	4.1	0.95		1.7	5.8
15	0	610	5.8	1.6		2.4	8.2
16	0	505	6.9	2.1		3.6	10.5
17	0	605	8.3	3.0		5.2	13.5
18	0	550	9.4	3.0		5.4	14.8
19	0	560	9.8	3.4		5.7	15.5
20	0	590	9.2	3.1		5.4	14.6
21	0	600	10.0	3.2		5.8	15.8
22	2.8	985	10.2	3.0	2.5	8.1	18.3
23	2.8	1010	9.7	3.3	2.4	7.7	17.4
24	0	790	12.2	3.9		6.5	18.7
25	0	685	14.3	4.6		8.3	22.6

Table 4

The Excretion of Conjugated Bile Acids in the Choledochostomy Drainage Bile of Patient (Case 1), and the Effect of Oral Administration of Desiccated Hog Bile

Days Postoperative	Hyodesoxycholic Acid Content of Hog Bile Administered	Concentration of Bile Acids in Drainage Bile			
		Total Bile Acids	Total Conjugated Bile Acids	Tauro-conjugated Bile Acids	Glyco-conjugated Bile Acids
	(millimoles per day)	(millimoles per liter)			
2	0	0	0		
3-4	0	trace	trace		
5-6	0	0.10	0.06	0.02	0.04
7-8	0	0.20	0.13	0.05	0.08
9-10	0	0.62	0.45	0.18	0.27
11	0	1.23	0.92	0.38	0.54
12	0	2.02	1.55	0.61	0.94
13	0	4.1	3.3	1.35	1.95
14	0	5.8	4.8	2.1	2.7
15	0	8.2	6.4	2.6	3.8
16	0	10.5	8.5	3.8	4.7
17	0	13.5	11.5	5.2	6.3
18	0	14.8	12.7	5.9	6.8
19	0	15.5	13.5	5.8	7.7
20	0	14.6	13.0	5.9	7.1
21	0	15.8	13.3	5.8	7.5
22	2.8	18.3	15.9	5.6	10.3
23	2.8	17.4	14.9	5.2	9.7
24	0	18.7	15.1	6.8	8.3
25	0	22.6	19.2	8.8	10.4

uous recirculation of bile in the hog was 600 mg. per kilogram of body weight[24]. There was no evidence of conversion of cholic acid to hyodesoxycholic acid in the hog. The accumulation of hyodesoxycholic acid during the course of continuous enterohepatic circulation in the hog can be described by means of an equation[24] which has the form of the "law of compound interest"; a similar equation can be applied to the disappearance of cholic acid or the conjugated cholates after a single oral administration to the hog.

Bile After Biliary Tract Surgery

Choledochostomy drainage of bile by means of a T tube frequently is established following surgery of the biliary tract, and often such drainage is maintained for periods which range from several weeks to several months. During such drainage periods only small amounts of bile, usually quite deficient in bile salts, enter the intestine[25b, 26]. A postoperative period of 10 to 20 days usually elapses before resumption of synthesis of bile acids by patients whose biliary tracts had been severely obstructed prior to operation.

As an illustration of fairly typical results, data are presented[25b] in tables 3 and 4 for the case* of a 58 year old white woman on whom a cholecystectomy was performed (case 1). This patient had experienced severe attacks of epigastric pain over a period of seven years prior to operation. At operation a contracted, thickened gallbladder was found with a 1.5 cm. stone imbedded in the fundus. The common duct was filled with a large putty-like mass, greenish-brown in color, which formed a cast of the common duct. This material was removed and a large T tube was placed in the common duct. After the operation, the patient's condition was satisfactory.

All of the various bile acids were practically absent from the drainage bile of this patient during the first four or five days after operation. The excretion of desoxycholic acid and of the total dihydroxycholanic acid fraction (which includes desoxycholic acid) was resumed somewhat earlier than the excretion of cholic acid. Cholic acid began to appear in the bile in measurable quantities on the ninth day.

In the case of this patient, the excretion of bile acids increased rather rapidly after the tenth day, but in other cases[25b] the

*From the service of Dr. Charles G. Johnston, Professor of Surgery, Wayne University College of Medicine, Detroit, Michigan.

resumption of bile acid synthesis was more retarded. The quantity of total dihydroxycholanic acids usually exceeded the amount of desoxycholic acid. This is evidence for the presence of one or more additional members of the dihydroxycholanic acid series. Inasmuch as Wieland and others[7a,9] have isolated chenodesoxycholic acid (anthropodesoxycholic acid) from gallbladder bile collected from cadavers, it is reasonable to assume that this dihydroxycholanic acid is a normal constituent of human bile, and that the difference between the values for the total dihydroxycholanic acids and those for desoxycholic acid corresponds to chenodesoxycholic acid.

The biliary excretion of this patient appeared to be approaching a steady-state at the time of termination of drainage, but it is doubtful whether the bile acid concentrations could be considered "normal" even at this time. However, the *relative proportions* of the various bile acids were rather constant after the twentieth day, with the exception of the two days on which desiccated hog bile was administered to the patient. The average proportions of cholic acid to desoxycholic acid to chenodesoxycholic acid for the twentieth, twenty-first, twenty-fourth, and twenty-fifth days were 3 : 0.98 : 0.74.

Appreciable fractions of the bile acids excreted by this patient during the first ten days after operation were unconjugated, but the percentage of the total bile acids in the conjugated form rose to 85 during the latter part of the period of drainage (table 4). The average ratio of glyco-conjugated to tauro-conjugated acids was 1.3 : 1 during periods when hog bile was not administered. Following the oral administration of dried gallbladder bile of the hog to this patient, the excretion of hyodesoxycholic acid (the predominant bile acid of hog bile) in the drainage bile commenced after one to two hours. Thereafter, the excretion of this "foreign" bile acid rose to a peak at about seven to ten hours after administration, then gradually declined; the total period of excretion comprised 18 to 20 hours in some cases. The average excretion of hyodesoxycholic acid was 88 per cent of the amount administered.

Replacement therapy

In table 5 data are presented which demonstrate that hyodesoxycholic acid of hog bile can enter the enterohepatic circulation

Table 5
The Enterohepatic Circulation of Glycohyodesoxycholic Acid
(Case 1)

Glycohyodesoxycholic Acid Content of Dried Hog Bile Administered	*Duration of Enterohepatic Circulation*	*Glycohyodesoxycholic Acid Content of Bile Excreted at End of Period of Circulation*	*Loss of Glycohyodesoxycholic Acid During Circulation*
(millimoles)	*(hours)*	*(millimoles)*	*(Per cent of initial amount)*
2.6	24	1.9	27
4.75	24	2.9	39
10.0	24	6.3	37
2.6	48	1.0	61.5
4.75	48	1.4	70.5
4.75	96	0.5	89.5

of patients to whom desiccated hog bile is administered orally. Similar experiments[25b] have shown that desiccated ox bile also can be efficiently used by patients with biliary deficiency. The oral administration of ox or hog bile to patients with choledochostomy drainage causes some stimulation of the *volume* of bile-flow, but the effect is less than that produced by comparable amounts of hydrocholeretics such as dehydrocholic acid. This smaller increase in volume probably is desirable when bile is administered preoperatively to patients with biliary obstruction. The difference between a choleretic and a hydrocholeretic agent is shown in table 6, which was derived from the experiments of Berman and others[27].

Loss of Bile Acids During Enterohepatic Circulation

During each passage of bile acids through the enterohepatic circuit some percentage of the total circulating bile acids is lost. This percentage varies from 10 to 20 per cent of the total. Schmidt and others[13] have investigated the fate of the bile acids which are lost during enterohepatic circulation, and they have concluded that, at least in the guinea pig, the disappearance is due to the oxidation of the bile acids to keto-bile acids in the cecum by the action of the microorganism, *Alcaligenes faecalis.*

Mayo and Greene[28] and Reiners[29] have concluded that, after the bile acids are absorbed from the intestine during enterohepatic circulation, the preferential route through which these acids reach the blood stream is by way of the lymphatics. The average concentration of bile acids in blood

Table 6
The Effect of Choleretic and Hydrocholeretic Agents on Biliary Excretion in Normal Animals

Stimulating Agent	*Percentage Increase*	
	Total fluids	*Total solids*
Ox bile salts (choleretic)	36	67
Dehydrocholic acid (hydrocholeretic)	106	63

of the peripheral circulation of normal animals is small (0.5 to 3 mg. per 100 ml. by various analytical methods), but in experimental animals and in patients with biliary obstruction the concentration increases many fold, and may even attain a value as high as 30 mg. per 100 ml. in peripheral blood[17a,24,25b,29,30]. In dogs the concentration of cholic acid in lymph of the thoracic duct rises rapidly following total biliary obstruction to values of 16 to 20 mg. per 100 ml. within 1 hour, and to peak values of 20 to 25 mg. per 100 ml. within four hours after onset of the obstruction[29].

The concentration of cholic acid in the peripheral blood of these experimental animals rises much more slowly (5 mg. per 100 ml. at 5 hours, and 16 mg. per 100 ml. at 13 hours after obstruction in one animal); but the concentration remains high for many days, and then begins to decline slowly after approximately 30 days of total obstruction[29]. This fall in the concentration of cholic acid in peripheral blood which occurs in chronic obstruction may be due to liver damage. Such damage to hepatic tissue from prolonged biliary obstruction may account for the observation that many patients with this condition exhibit only moderate elevation of the concentration of bile acids in the peripheral circulation[25b,29].

Bile acid tolerance tests[30b,31] may prove to be useful from a diagnostic standpoint in such cases. Baker and others[31] have conducted comparative studies of bile acid tolerance tests with the bromsulphthalein excretion test for liver function in experimental cases of central necrosis of the liver produced by carbon tetrachloride. In early central necrosis the bromsulpthalein excretion was definitely delayed, while cholate clearance was only slightly changed from the normal. These investigators obtained evidence for a competitive effect when cholate and bromsulpthalein were injected simultaneously; this effect consisted of a retardation in bromsulphthalein excretion under these conditions. A similar effect was reported by Mills and Dragstedt[32]. Although the early effect of carbon tetrachloride poisoning on the excretion of *injected* cholates is small, the effect of this compound, and of chloroform and tetrachlorethane, upon bile acid synthesis is large[14a].

Functions of Bile Acids

The most important known functions of the bile acids are: the acceleration of the intestinal digestion of lipids through emulsification and through direct catalysis of the hydrolysis of fats by pancreatic lipase[33]; and the promotion of the intestinal absorption of fats[34] and of the fat-soluble vitamins or provitamins: carotene[1a,e,35], vitamin D[36], vitamin E[37], and vitamin K[1f,c,38]. The effect on the intestinal absorption of vitamin K is particularly important from a clinical standpoint, inasmuch as the oral administration of bile and vitamin K to patients with biliary obstruction or biliary fistulas can alleviate the bleeding tendency in such cases by promoting the restoration of plasma prothrombin concentrations to normal levels[1b,c].

The solubility of cholesterol in aqueous solutions of bile salts[39] in contrast to the insolubility of this sterol in water alone provides an explanation for the fact that under normal conditions considerable amounts of cholesterol are excreted in bile without precipitation in the gallbladder and bile ducts. Other factors also, such as the presence of proteins in bile, may contribute to the solubility of cholesterol in this fluid. The low bile salt concentrations frequently found in bile from damaged gallbladders[40] have suggested the possibility that bile salt—cholesterol ratios might be correlated with the formation of cholesterol stones in the biliary tract. Ravdin and others[26b] have pointed out that there are difficulties in attempting such correlations, but, nevertheless, they believe that low concentrations of bile salts may be an important factor in the etiology of gallstones. The physiologic and pathologic aspects of gallbladder function are beyond the scope of the present review, but much information of interest is summarized in the paper of Ravdin and colleagues[26b].

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"The prime object of the medical profession is to render service to humanity; reward or financial gain is a subordinate consideration. Whoever chooses this profession assumes the obligation to conduct himself in accord with its ideals."—Principles of Medical Ethics of the American Medical Association, Chapter 1, Section 1.

APRIL, 1952

THE MACNIDER MEMORIAL ISSUE

This issue of the *North Carolina Medical Journal* is presented to its readers as a reminder of the life and works of William deBerniere MacNider. The portrait was painted in 1945 by John C. Johansen and hangs in the medical library of the University of North Carolina at Chapel Hill. Dr. George's summary of Dr. MacNider's career was presented as a memorial to the general University faculty on October 26, 1951. The papers were contributed by nine of his colleagues of the basic science faculty of the medical school in Chapel Hill. Each of the contributors has attempted to present a summary of progress in the area of his particular interest.

It should be remarked that several of the papers describe areas of research which are in great ferment today, areas to which Dr. MacNider contributed significantly in past years. One wonders whether this prescience, choosing years in advance to work on problems of the future, is not another measure of the remarkable man who has left our midst.

Today North Carolina appears about to blossom as a medical center, with a higher ratio of first-class medical schools to population than any similar division of the republic. In this context we should not forget that Dr. MacNider was its first bloom—North Carolina's first native medical statesman.

—John Borden Graham, M.D.

* * *

DR. WILLIAM DEBERNIERE MACNIDER*

The daily papers announced that on May 31 Dr. William deB. MacNider died in a Durham hospital. Because he so loved life, and got so much from life, it is hard to believe that he is no longer with us in the flesh.

Dr. MacNider began his teaching career at the University of North Carolina while he was still an undergraduate student. This was quite in keeping with his character, for all the rest of his life he continued to be both a teacher and a student. He was almost certainly the most famous medical man that North Carolina ever produced. His research made him an international authority on the kidney. The long list of his achievements in *Who's Who* is evidence of the wide recognition given him at home and abroad. Although he walked with the kings of medicine, however, he never lost the common touch. His capacity for friendship seemed unlimited.

Dr. MacNider was graduated in 1903 from the University medical school during its brief life as a four year school. It is not too much to say that the quality of the work done by him as professor of pharmacology, as well as his constant research, has done much to keep alive in the University two year school of medicine the high standards of medical teaching and research that made possible its expansion into a four year school. His legion of friends rejoice that he lived to see this lifelong dream realized. Nothing better could be wished for the full grown University of North Carolina School of Medicine than that it may retain Bill MacNider's enthusiasm as teacher and as student.

*Reprinted from the North Carolina Medical Journal for June, 1951 (volume 12, page 241).

DOCTORS IN POLITICS

An editorial in the *News and Observer* for March 14, headed "Doctors Lost," said in part:

"One interesting feature of the New Hampshire presidential primary is that it not only marked a defeat for old-time politicians, it was also a setback for organized doctors . . . doctors as a group are showing more and more disposition to take sides in every political campaign with or without a reason —or even an excuse . . . the organized doctors are reported to have 'gone down the line' for Senator Taft in the New Hampshire primary. Presumably, if they follow their national leaders they will do the same thing in other states.

"If the doctors of this country want to identify themselves with one political party and with one faction of that party, that is their business. But, they should do so openly and as politicians, not as doctors. And, as politicians they must expect to pay the same penalty as other politicians if and when they lose."

Evidently the editor did not trouble to get the facts before expressing an opinion. The executive secretary of the New Hampshire Medical Society writes that while there was a doctors' committee for Taft of about 40 members, there was no organized statewide activity. An equally large number of doctors favoring the candidacy of General Eisenhower were also associated but not as an organized committee. As a matter of fact, Dr. Robert O. Blood of Concord, favorable to General Eisenhower, was elected a delegate to the Republican National Convention and received the second highest total number of votes in the Republican primary.

"Any charge that the New Hampshire Presidential primary marked a defeat for old-time politics and a setback for organized doctors is entirely untrue. In the first place, there was no such factor as an organized doctors group and New Hampshire's men of medicine did not go down the line for Senator Taft, although it was recognized the Senator has done a great deal for organized medicine in his Senate actions aimed at defeating socialization. It can best be stated that New Hampshire doctors were during the primary and are now very much interested as individuals in political activity insofar as it is aimed at furthering the projection into office of men of integrity, ability and common sense, and many New Hampshire doctors have told me they would consider both Senator Taft and General Eisenhower to have these qualities."

". . . I would like to reiterate that New Hampshire doctors have a high regard for both Senator Taft and General Eisenhower and there was definitely no 'down the line' effort on the part of New Hampshire's men of medicine for any candidate during the Presidential Preference Primary."

It is high time that doctors, as well as all other intelligent citizens, were getting interested in political activity aimed at electing "men of integrity, ability and common sense." No doubt many readers of this journal recall an editorial in the *Journal of the American Medical Association*, based in turn upon one from a Dallas paper, in which it was shown that in the 1948 election only 36 per cent of doctors and 38 per cent of lawyers voted, whereas 85 per cent of laborers and 90 per cent of government employees went to the polls.

Doctors are noted for being individualists, and it is doubtful if any profession can claim a larger proportion of independent voters. If, however, taking an active interest in "the projection into office of men of integrity, ability and common sense," regardless of their party affiliation, be treason — then make the most of it!" The hope of obtaining good government by competent men of integrity lies in a greatly increased number of independent voters and in a greatly increased interest in politics on the part of good citizens everywhere.

* * *

POSTGRADUATE OPPORTUNITIES IN NORTH CAROLINA

North Carolina doctors have long been fortunate in the opportunities afforded them for postgraduate training. More than 30 years ago the University of North Carolina pioneered in offering extension courses to doctors throughout the state. From its early days, Duke has given excellent symposiums every fall for the doctors of the state. More recently the faculty of Bowman Gray has begun to give postgraduate courses in cardiovascular diseases and in neurology and psychiatry, sponsored by the State Board of Health. Excellent programs are arranged by other medical organizations in the state: for example, the annual symposiums of the Raleigh Academy of Medicine, the Greensboro Academy of Medicine, the Watts Hospital staff, the New Hanover County Medical Society, and the Matheson and the Nalle Lectures in Charlotte. An excellent example of North Carolina postgraduate education is to be found on page 216 of this issue—the annual postgraduate course given by the Duke medical faculty, which this year will be devoted to pediatrics.

Medicine more than any other profession demands that its members remain students as long as they are in practice. North Carolina doctors have no valid excuse for not keeping up with the advances along the medical front.

Committees and Organizations

North Carolina Tuberculosis Association

Program of Annual Meeting
Sir Walter Hotel, Raleigh, North Carolina
April 23-24

Wednesday, April 23, 1952

9:30 A.M.—Registration

1:30 P.M.—**General Session**
- Presiding: Roland L. Garrett, President North Carolina Tuberculosis Association
- Invocation:
- Welcome:
- Business Meeting of the Association
- The Voluntary Health Agency in the American Scheme — James G. Stone, Executive Secretary National Tuberculosis Association

2:45 P.M.—**Medical Section**
- Presiding: E. E. Menefee, M.D., Vice President North Carolina Trudeau Society
- A New Test for Viability for Tubercle Bacilli — A. M. Vandiviere and W. Harold Gentry, M.D., North Carolina Sanatorium
- X-Ray Aspects of Segmental Anatomy — George J. Baylin, M.D., Duke University School of Medicine
- Indications for Thoracic Surgery — J. D. Murphy, M.D., Veterans Administration Hospital, Oteen
- The Results of Thoracic Surgery in Treatment of Certain Types of Pulmonary Tuberculosis—William C. Sealy, M.D., Duke University School of Medicine

2:45 P.M.—**Non-Medical Section**
- Presiding: Mrs. J. J. Rowland, President, Lenoir County Tuberculosis Association
- A Critical Evaluation of Mass Roentgen Surveys—David A. Cooper, M.D., President-elect American Trudeau Society
- Case Finding in Industry and General Hospitals
  - Discussion Leader: Mildred T. Greene, Executive Secretary Greensboro Tuberculosis and Heart Associations
  - Other speakers to be announced
- Plans for Tuberculosis Education, Research and Service at the University of North Carolina — Dr. Elizabeth L. Kemble, Dean, University of North Carolina School of Nursing

5:00 P.M.—Board of Directors Meeting, North Carolina Tuberculosis Association

7:00 P.M.—**Dinner Meeting—Medical Section**
- Presiding: R. B. C. Franklin, M.D., President North Carolina Trudeau Society
- Mycotic Lung Infections — David T. Smith, M.D., Duke University School of Medicine
- Diabetes and Tuberculosis—David A. Cooper, M.D.

7:00 P.M.—**Dinner Meeting—North Carolina Conference of Tuberculosis Secretaries**

Thursday, April 24, 1952

9:30 A.M.—**General Session**
- Presiding: E. N. Pope, President Wake County Tuberculosis Association
- Trends in Public Relations — A. L. Bechfold, Director, Public Relations, Lance, Inc., Charlotte
- Objectives of the Buncombe County Come Back Club—Mrs. I. G. Craig, Asheville
- Symposium — Reports of Official Agencies
  - North Carolina Sanatoriums—H. Stuart Willis, M.D., Superintendent North Carolina Sanatoriums
  - North Carolina Health Department—J. W. R. Norton, M.D., State Health Officer
  - North Carolina Welfare Department — Dr. Ellen Winston, Commissioner North Carolina State Board of Public Welfare
  - Tuberculosis of the Mentally Ill —Edward N. Pleasants, M.D., Superintendent State Hospital at Raleigh

12:30 P.M.—**Luncheon Meeting**
- Presiding: Roland L. Garrett
- Invocation: The Reverend Dr. T. A. Cheatham, Minister Emeritus, Pinehurst
- 1951 Christmas Seal Sale Report—W. C. Jackson, M.D., State Chairman
- Presentation of Speaker—Mrs. Baxter Durham, Executive Secretary Wake County Tuberculosis Association
- A Vision Plus a Task — Mrs. Earl Brian, Raleigh

New Scrub-Up Outdoes Soap in Clinical Trial
By Army M.D.'s

A new surgical scrub technique recommended for the operating theater under combat conditions because it hastens pre-operative procedures has just been reported by a team of Army doctors. Employing a preparation known as pHisoHex, the technique reduces by 70 per cent the time required to cleanse both the surgeon's hands and the site of the operation, or wound.

"The use of this technique greatly simplifies and hastens the pre-operative procedure" and "offers continued protection during an operation," according to Major Curtis P. Artz, Col. Edwin J. Pulaski, and Col. Joseph R. Shaeffer of the Brooks Army Hospital, Ft. Sam Houston, San Antonio, Texas, who reported their findings in the U. S. Armed Forces Medical Journal.

pHisoHex is a soapless sudsing detergent fortified with a potent nonirritating antiseptic, hexachlorophene, in the past popularly called "G-11." It is produced by Winthrop-Stearns, Inc., pharmaceutical manufacturers.

Cost of scrubbing was reduced from 17 cents to 3 cents. The doctors also proved clinically that a 3-minute scrub with pHisoHex is as effective in killing germs as the 10-minute "routine scrub" with soap, water and brush, long widely used but frequently considered to be the cause of skin irritation and soap-sensitivity.

BULLETIN BOARD

DUKE MEDICAL SCHOOL POSTGRADUATE COURSE

The next Postgraduate Course of the Duke University School of Medicine will be held on June 16-19. With the exception of Dr. Ralph Victor Platou, professor of pediatrics and chairman of the Department of Pediatrics, Tulane University School of Medicine, New Orleans, Louisiana, instructors for the course will be drawn from the faculty of the Duke University School of Medicine.

Program

Monday, June 16

8:30 a.m. Registration
9:00 a.m. "Prenatal Influences"—Dr. Ralph Victor Platou, Tulane University School of Medicine, New Orleans, Louisiana
10:00 a.m. "Congenital Heart Disease" — Dr. Jerome S. Harris, Professor of Pediatrics
11:00 a.m. "Prematurity" — Dr. Angus McBryde Associate Professor of Pediatrics
12:00 noon Lunch
2:00 p.m. Ward rounds or visits to the various clinics
4:00 p.m.
7:30 p.m. Round-Table Discussion: "Accidents in Children" — Moderator, Dr. Platou; Discussants, Dr. Barnes Woodhall, Professor of Neurosurgery, and Dr. Jay Morris Arena, Associate Professor of Pediatrics

Tuesday, June 17

9:00 a.m. "Birthmarks and Moles"—Dr. Platou
10:00 a.m. "Common Plastic Surgical Problems in Children"—Dr. Kenneth L. Pickrell, Professor of Plastic Surgery
11:00 a.m. "Some Dermatological Problems in Children" — Dr. J. Lamar Callaway, Professor of Dermatology and Syphilology
12:00 noon Lunch
2:00 p.m. Ward rounds or visits to the various clinics
4:00 p.m.
7:30 p.m. Round-Table Discussion: "Epilepsy" — Moderator, Dr., Platou; Discussants, Dr. Hans Lowenbach, Associate Professor of Psychiatry; Dr. Guy L. Odom, Professor of Neurosurgery; and Dr. E. Charles Kunkle, Associate Professor of Medicine in Charge of Neurology

Wednesday, June 18

9:00 a.m. "The Tuberculous Child"—Dr. Platou
10:00 a.m. "The Allergic Child"—Dr. Susan Dees, Associate Professor of Pediatrics
11:00 a.m. "The Problem Child" — Dr. Leslie B. Hohman, Professor of Psychiatry
12:00 noon Lunch
2:00 p.m. Ward rounds or visits to the various clinics
4:00 p.m.
7:00 p. m. Guests and faculty are invited to Turnage's for a barbecue dinner

Thursday, June 19

9:00 a.m. "Cancer in Children"—Dean Wilburt C. Davison, Professor of Pediatrics and Chairman of the Department of Pediatrics
10:00 a.m. "Congenital Disturbances of Bone Formation"—Dr. Platou
11:00 a.m. "Common Pediatrics Problems" — Dr. Arthur Hill London, Associate in Pediatrics
12:00 noon Lunch
2:00 p.m. Ward rounds or visits to the various clinics
4:00 p.m.

NEWS NOTES FROM THE DUKE UNIVERSITY SCHOOL OF MEDICINE

Dr. William deMaria, instructor in pediatrics at the Duke University Medical School, recently received a $30,000 research grant from the John and Mary R. Markle Foundation of New York.

Dr. deMaria, one of six pediatricians awarded grants this year, became the fourth Duke pediatrician to win a five year Markle grant.

Dr. deMaria is working chiefly with heart and kidney diseases in children. The Duke Medical School will administer and supplement the grant ($6,000 a year for five years).

Other Duke pediatricians already working under Markle grants are Dr. Ivan W. Brown, Jr., Dr. Samuel P. Martin, and Dr. George W. Schwert, Jr. The Duke doctors are among 87 researchers from 46 medical schools who have received such grants since the Markle program was started in 1948.

NEWS NOTES FROM THE UNIVERSITY OF NORTH CAROLINA SCHOOL OF MEDICINE

Dr. Lyle Weed, from the Section on Bacteriology of the Mayo Clinic, spoke to the faculty and student body on "Pulmonary Lesions of Varied Etiology: The Importance of Cultures of Surgical Specimens" on April 19.

* * *

Dr. K. M. Brinkhous, professor of pathology, was guest speaker at the March meeting of the Moore County Medical Society in Pinehurst; his topic was "Hemorrhagic Diseases."

* * *

Dr. John B. Graham, of the Pathology Department, attended a meeting of Markle Scholars held in conjunction with the Pre-professional Conference at Buck Hill Falls, Pennsylvania, on April 6 through April 9.

* * *

"The Influence of Fluorine on Calcification" was the subject of a paper by Mr. M. K. Berkut, of the Department of Biological Chemistry, presented before the International Association for Dental Research in Colorado Springs in March.

* * *

The American Heart Association has made a grant of $9,000 to Dr. C. Bruce Taylor, associate professor of pathology, for a study entitled "Evaluation of Macacus Rhesus Monkey as Experimental Animal for Study of Atherosclerosis (including studies on cholesterol metabolism using C_{14} labeled acetate)."

* * *

Dr. Charles H. Burnett, professor of medicine, was guest speaker at the staff conference of the U. S. Naval Hospital at Camp Lejuene on March 11; he spoke on "The Evaluation of Thyroid Function and the Medical Treatment of Hyperthyroidism." He took part in a roundtable discussion on "Diabetes," and discussed "Renal Complications of Pregnancy," at the Third Annual Postgraduate Day Exercises of the Memorial and Crippled Children's Hospital in Roanoke the latter part of March.

* * *

Dr. Isaac M. Taylor, of the Department of Medicine, spoke to the Watts Hospital medical staff on "Metabolic Factors Affecting Electrolyte Distribution Within the Body" in March.

* * *

Dr. George D. Penick, Instructor in Pathology, presented a paper at the Symposium on Irradiation Damage at the American Association of Pathologists and Bacteriologists in New York City on April 11.

* * *

At the April meetings of the Federation of American Societies for Experimental Biology in New York, the following staff members presented papers:

Robert D. Langdell, Robert H. Wagner, and K. M. Brinkhous—"Antihemophilic Factor: its Effect on One-Stage Clotting Tests as the Basis for a Simple AHF Assay Procedure." (Department of Pathology)

K. M. Brinkhous, F. C. Morrison, Jr., and M. E. Murrer—"Comparative Study of Clotting Defects in Human, Canine and Procine Hemophilia." (Department of Pathology, University of North Carolina, and Department of Agricultural Chemistry, University of Missouri.)

Fred W. Ellis — "Observations on the Bronchodilator Activity of Visammin (Khellin)." (Department of Pharmacology)

M. K. Berkut and James C. Andrews—"Influence of Fluorides on Mechanism of Tooth Development." (Department of Biological Chemistry and Nutrition)

J. Logan Irvin and Elinor Moore Irvin—"Interaction of Quinacrine with Nucleic Acids and with their Products of Depolymerization by Alkali and by Enzymes." (Department of Biological Chemistry and Nutrition)

W. R. Straughn—"Quantitative Determination of Low Concentrations of Nitrate by Enzymatic Reduction." (Department of Bacteriology)

Jessica H. Lewis and John H. Ferguson—"Inhibition of Fibrinolysin and Trypsin." (Department of Physiology)

John H. Ferguson, G. A. Andrews, and Marshall Brucer—"Blood-clotting Studies Following Internal Radiation." (Physiology Department, University of North Carolina, and the Oak Ridge Institute of Nuclear Studies)

Edwin P. Hiatt and Inez Greene—"Distribution, Excretion and Metabolism of the Nitrate Ion Administered Intravenously as $NaNO_3$." (Department of Physiology)

Brodie C. Nalle Lecture†

The third Brodie C. Nalle Lecture will be presented by the Brodie C. Nalle Fund of The Nalle Clinic Foundation on Friday, April 25, 1952, at 8:00 p.m. in the ballroom of the Hotel Charlotte, Charlotte, North Carolina. The speaker for this lecture will be Dr. Richard W. TeLinde of Johns Hopkins University School of Medicine, Baltimore, Maryland. His subject will be: "Carcinoma of the Cervix: Recent Advances in Diagnosis and Treatment."

Dr. TeLinde is professor of gynecology at Johns Hopkins University School of Medicine. He has been particularly interested in his subject for many years, and is considered an authority on this disease. He is a member of the American Gynecological Society, the American Gynecological Club, Southern Surgical Society, Southern Medical Society, and the American Medical Association. He also has honorary memberships in the Peruvian Gynecological Society and the Brazilian Gynecological Society. Dr. TeLinde is also author of the textbook, "Operative Gynecology."

†Dr. Nalle died of coronary thrombosis on February 13, 1952.

North Carolina Surgical Association

The North Carolina Surgical Association held its regular spring meeting at Sedgefield Inn on March 8. The following scientific program was presented: "Endometriosis — Its Pathological Physiology and Surgical Therapy"—Dr. William W. Noel of Henderson; "The Management of Traumatic Abdominal Injuries"—Dr. George W. Joyner of Asheboro; and "The Present Day Management of Burns with Special Reference to Early Grafting"—Dr. James F. Marshall of Winston-Salem. The one day meeting was concluded by a golf tournament and a formal dinner.

American Cancer Society, North Carolina Division, Inc.

Ready for loan to county medical societies or schools of medicine is the new medical film of the American Cancer Society and the National Cancer Institute, "Uterine Cancer: The Problem of Early Diagnosis." The film may be borrowed from State Headquarters, American Cancer Society, North Carolina Division, Haynes Building, Mt. Airy, North Carolina.

In 16 mm., with color and sound, the film may be shown in 21 minutes. The **Journal of the American Medical Association** of February 2, 1952, describes the film fully and comments: "The picture is well organized. The photography, animation, and narration are excellent, and the film is highly recommended for the general practitioner, the advanced medical student and hospital meetings."

* * *

Two North Carolina medical men attending the Second National Cancer Conference at Cincinnati on March 3, 4, and 5 were Dr. L. H. Campbell of Asheville, director of the Buncombe County Cancer Center, and Dr. Robert F. Young, health officer for Halifax County. In reporting on the sessions, in which 400 cancer specialists and researchers participated, Dr. Young commented: "All of us came away feeling that we are knocking on the door of the answer to the cause of cancer. We left with great encouragement and hope."

* * *

Looking forward to the 1952 Cancer Crusade in April, 10 district meetings for volunteer workers of the North Carolina Division, ACS, were held March 3-14. They were held in Lenoir, Asheville, High Point, Charlotte, Lumberton, Burlington, Tarboro, Clinton, Washington, and Elizabeth City. Physicians who are working in, or have worked in, the detection centers of the state were speakers at eight of the sessions.

North Carolina Alcoholic Rehabilitation Program

A wide range of topics delivered by a staff of prominent lecturers will be featured at North Carolina's pioneer Summer Studies on Facts About Alcohol in Chapel Hill June 9-13, it was announced recently by S. K. Proctor, director of the North Carolina Alcoholic Rehabilitation Program.

Sponsored jointly by the ARP and the University of North Carolina Extension Division, under the direction of Russell M. Grumman, the Summer Studies will present a teamwork approach toward the social, medical, religious, and educational problems of alcohol and the illness of alcoholism.

To supplement the varied lecture program, the Studies will feature special afternoon seminars on community problems. They will offer techniques of successful pastoral counseling, the latest methods

of unbiased instruction about alcohol, and the relation of alcoholism problems to courts, jails, and the social agency. One seminar will feature the administrative and therapeutic aspects of the ARP.

Special films to be shown and discussed include **Preface to a Life, Overdependency, Alcohol and the Human Body,** and **Alcoholism.** An information folder describing the fees, matriculation, curriculum and staff of the Studies is available at The ARP, Box 9118, in Raleigh.

* * *

Twenty scholarships to the 1952 Yale University Summer School of Alcohol Studies are now available to North Carolina teachers, physicians, public health and social workers, ministers, personnel workers, law enforcement officials, Salvation Army workers, Alcoholics Anonymous members, and private citizens interested in the latest facts on alcohol problems.

Sponsored by the North Carolina Alcoholic Rehabilitation Program, the special educational grants this year include tuition, room, and board, it was announced by S. K. Proctor, executive director of the ARP. Sunday meals and travel expenses are not included.

Applications for the special scholarships must be submitted by letter to the Raleigh office of the ARP by April 25, and should include a statement of professional background and any particular interest in newly discovered facts and problems relative to alcohol and the illness of alcoholism.

All requests for further information on the scholarships and for the Yale School pamphlet should be addressed to S. K. Proctor, Box 9118, Raleigh, N. C.

Edgecombe-Nash Medical Society

The regular monthly meeting of the Edgecombe-Nash Medical Society was held on March 12 at the New Ricks Hotel. Speaker of the evening was Dr. George C. Ham, professor of psychiatry of the University of North Carolina School of Medicine. The Society welcomed into its membership Dr. Harold B. Spangler, who recently opened an office in Rocky Mount for the practice of pediatrics.

Halifax County Medical Society

"What the General Practitioner Should Know About Glaucoma" was the topic of a discussion by Dr. Luther C. Brawner of Richmond, Virginia, at the regular monthly dinner meeting of the Halifax County Medical Society in Roanoke Rapids on March 14.

News Notes

The North Carolina Cancer Institute and the North Carolina Division of the American Cancer Society were hosts at the official opening of the Institute (a nursing home providing terminal care for needy cancer patients) at Lumberton on Sunday, March 23.

* * *

Dr. Thomas R. Huffines and Dr. George G. Gilbert have announced the association of Dr. Ralph G. Eaker in the practice of urology in Asheville.

* * *

Dr. Charles M. Kendrick and Dr. Frederick A. Thompson, Jr., of Lenoir, have opened their office as partners in the practice of internal medicine.

* * *

Dr. John S. Rhodes and Dr. Tom B. Daniel have announced the removal of their offices to 700 West Morgan Street in Raleigh.

General Practice Postgraduate Training

The General Practice Group of the University of Tennessee has established a postgraduate clinical training program for general practitioners. This has been approved by the American Academy of General Practice for its members.

The program is designed for the general practitioner on an individual basis, according to his individual needs. One week to one month of training is offered.

Each doctor will spend morning hours in his choice of any one of the University specialty fields. This will be active work at the resident level. The afternoons will be spent in the General Practice Clinic, where the medical students get active general practice experience. Evenings are utilized in the emergency room of the John Gaston Hospital, which is supervised by members of the General Practice Staff.

General practitioners who would like to participate or who desire further information, may write to the General Practice Office, University of Tennessee, Memphis, Tennessee. There is no fee charged for this training.

News Notes from the American Medical Association

A request for "cooperation" on the part of the American Medical Association in making various health and medical care statistics available to the President's Commission on the Health Needs of the Nation was approved by the board of trustees.

Dr. George F. Lull, secretary and general manager, officially replied to the Commission, saying: "It has long been the policy of the American Medical Association to permit all survey and study groups to have access to the medical data in its possession. The Board's action, however, should not be construed as implying approval of the Commission or its projected program which we believe to be of political intent."

* * *

Dr. Gunnar Gundersen of Wisconsin, a member of the A.M.A. Board of Trustees, recently was elected chairman of the Joint Commission on Accreditation of Hospitals. The Commission recently selected Edwin L. Crosby, M.D., as director.

* * *

More than 500 medical and farm leaders gathering in Denver for the Seventh Annual Conference on Rural Health made news. The Conference's theme —how to help rural communities help themselves to better health—was carried not only in many newspapers and magazines but also to a large radio audience in the Denver area and throughout the midwest.

* * *

A $39,650 budget to wind up activities of the Committee for the Survey of Medical Education has been approved by the A.M.A. Board of Trustees. The Committee reports that Survey findings should be completed and sent to the publisher by June, 1952.

A representative sample of 41 medical schools was studied with the idea of pointing up the basic problems facing medical education today. Broad objectives of the Survey are: (1) to improve medical education to meet over-all health needs of the public; (2) evaluate the degree to which medical schools are meeting the need for physicians; (3) promote the advancement of medical science, and (4) inform the public of the nature, content and purposes of medical education.

* * *

The A.M.A. plans to centralize its medical socio-economic files in the near future. At its last meeting, the Board of Trustees authorized the appointment of an assistant librarian to assemble and catalogue this material from the various councils, committees, and bureaus of the Association. Eventually a complete history of A.M.A. policies and attitudes will be compiled from this source.

* * *

The A.M.A. Board of Trustees recently appointed a liaison committee of Doctors Elmer Henderson, chairman, Perrin H. Long, George F. Lull, Henry B. Mulholland, Harvey B. Stone, and Walter B. Martin, to confer with the American Legion on matters dealing with national health and medical care problems. On March 1, this group met with the American Legion's Committee on Rehabilitation in Washington, D. C. The committee will meet again in April with Legion representatives.

* * *

The A.M.A. has pledged continued financial support to the Commission on Chronic Illness in the amount of $80,000 to be paid over a four-year period. The Commission, an independent national agency, is conducting an intensive study of chronic illness—one of the most important health problems in America today. This year's installment of $20,000 was recently turned over to the Commission. A $300,000 budget set by the Commission for the coming four-year period has been met by twelve contributing organizations.

* * *

A new series of health education radio programs, called "Medicine, U.S.A.," are being carried over the National Broadcasting Company network on six successive Saturdays—the first on March 29. Sponsored by the American Medical Association and county medical societies in cooperation with NBC, the shows are broadcast from New York at 7:30 p.m., EST. Charles Laughton, noted English character actor, narrates the series.

Subjects include: March 29—"Alcoholism;" April 5—"Psychiatry;" April 12—"Longer Life;" April 19—"Contagious Diseases;" April 26—"Exercise and Athletics," and May 3—"Medicine's Progress."

John and Mary R. Markle Foundation

Markle Fund Grants for Medical School Scientists

Twenty-one young scientists, all medical school faculty members, have been named as the fifth group of Scholars in Medical Science by the John and Mary R. Markle Foundation. This is the largest number appointed for any year since the program began in 1948, John M. Russell, executive director of the fund, announced. With total appropriations of over $2,500,000 for this program, the Foundation is now making grants toward the support of 87 doctors in 49 medical schools.

The purpose of the program is to help relieve the shortage of medical school teachers and investigators by providing academic security and financial assistance for young faculty members early in their careers. All grants are made direct to the medical schools at the rate of $6,000 annually for five years, and are earmarked for support of a specific scholar and his research.

Among the scholars whose appointments begin in 1952 is Dr. William J. A. DeMaria, instructor in pediatrics, Duke University School of Medicine.

Life Insurance Medical Research Fund

Life insurance companies of the United States and Canada will give more than $780,000 this year for research in heart disease, it was announced recently by M. Albert Linton, chairman of the Board of the Life Insurance Medical Research Fund.

The grants being made to medical schools and to individual scientists for heart disease research this year bring to $4,700,000 the total amount of money given by the life insurance business since the Life Insurance Medical Research Fund was organized late in 1945. The Fund is supported by the annual contributions of 141 United States and Canadian companies.

Included in the grants are two to Duke University: $14,580 for research by Dr. Philip Handler on humoral relationships in hypertension; and $22,140 for research by Dr. E. A. Stead, Jr., on cardiovascular and respiratory physiology, and organ metabolism.

(BULLETIN BOARD CONTINUED ON PAGE 220)

Classified Advertisements

THE MOUNT SINAI HOSPITAL
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Announces its Second Annual Seminar on
RECENT ADVANCES IN DIAGNOSIS AND TREATMENT

May 22, 23, 24, 1952

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Dr. D. M. Bergenstal, University of Chicago, Surgery of the Adrenal.
Dr. Wm. Dameshek, Tufts Medical College, Advances in Hematology.
Dr. D. C. Darrow, Yale University, Electrolyte Disturbances.
Dr. R. Elman, Washington University, Recent Advances in Surgery.
Dr. J. W. Hinton, New York University, Advances in Gastric Surgery.
Dr. J. B. Kirsner, University of Chicago, Gastrointestinal Diseases.
Dr. R. Levine, Michael Reese Hospital, Adreno-Cortical Steroids; also Recent Advances in Diabetes.
Dr. J. H. Means, Harvard University, Advances in Thyroidology and Clinical Applications.

Session to be held at the Delano Hotel, Miami Beach Florida.

Panel Discussion will follow each Session.

Registration Fee $20.00 (Cocktail Party, Banquet $7.50 optional.)

Address:
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Mount Sinai Hospital of Greater Miami
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BULLETIN BOARD

(CONTINUED FROM PAGE 219)

Michael Reece Hospital Postgraduate School

The Michael Reese Hospital Postgraduate School is offering five postgraduate courses during April and May. The first course—"Surgery—Indications, Pre- and Post-Operative Care"—was held from April 14 to 18. Other courses will be given as follows: "Clinical Dermatology—Refresher Course in Diseases of the Skin for General Practitioners," April 21 to 25; "Diseases of the Endocrines—Physiology and Diagnostic Methods," April 28 to May 9; "Recent Advances in Internal Medicine," May 12 to 24; "Recent Advances in Pediatrics — Diagnostic and Therapeutic Measures," May 26 to 31. For further information on any of these courses, address: Dr. Samuel Soskin, Dean, 29th Street and Ellis Avenue, Chicago 16, Illinois.

University of Michigan Fifth Annual Conference on Aging

"Housing the Aging" is the topic for the University of Michigan Fifth Annual Conference on Aging to be held in Ann Arbor, Michigan, July 24-26, 1952. The three-day conference will consider the housing needs of healthy, chronically ill, confused, and disabled older people living in urban and rural areas. Among the topics to be discussed are types of housing and living arrangements; architectural designs and costs; hygiene and safety standards; social and economic aspects of housing; and auxiliary services. The conference is designed to serve as a forum for interchanging information and for getting action on the difficult problem of financing housing for the aging.

The conference is under the co-sponsorship of the Institute for Human Adjustment, Schools of Architecture, Business Administration, Social Work, Public Health, and the Medical School, Extension Service and Summer Session of the University of Michigan; the Michigan State Medical Society; the Committee on Aging and Geriatrics of the Federal Security Agency, Washington, D. C., and the Housing and Home Finance Agency, Washington, D. C.

Conference registration materials may be obtained by writing to Dr. Wilma Donahue, Institute for Human Adjustment, Room 1510, Rackham Building, Ann Arbor, Michigan.

Committee on Medical Motion Pictures

The Committee on Medical Motion Pictures of the American Medical Association has completed the 1951 supplement to the second revised edition of the booklet entitled **Reviews of Medical Motion Pictures.** This supplement contains 90 reviews of medical and health films reviewed in The **Journal of the American Medical Association** from January 1, 1951, through December 31, 1951. Each film has been indexed according to subject matter. The purpose of these reviews is to provide a brief description and an evaluation of motion pictures which are available to the medical profession.

Copies have been sent to the secretary of each of the state medical societies. Complimentary copies will be sent to county medical societies and other medical organizations upon request, from:

Committee on Medical Motion Pictures
American Medical Association
535 North Dearborn St.
Chicago, Illinois

Society for the Prevention of Asphxial Death

The Society for the Prevention of Asphyxial Death has announced the beginning of its sixth year of sponsorship of courses in resuscitation. March 7-8, 1952, it conducted its sixty-third class at its headquarters in the Academy of Sciences Building, 2 East 63 St., New York City. The April class met April 4-5. The May class will meet May 4-5, and the June class on the 6 and 7.

Instruction stresses the elimination of the death zone of the respiratory tract through lectures, demonstrations, and clinical practice in laryngoscopy and intubation of dogs and cadaver.

It is recognized that this effort, viewed from the over-all field of medical education, is but a pilot project, nevertheless it is believed that it has helped to fill the gap in essential information which medical and dental students presently face. It is hoped that the medical and dental schools over the country will shortly provide this integrated information, making it a commonplace in graduate instruction.

While the course is given regularly in New York City, the first Friday and Saturday of each month, out of town classes have met in Boston, Massachusetts; Colombus, Ohio; Washington, D. C.; Los Angeles, California, and in Honolulu. Requests for out of town courses will be considered where application is made by a group of at least 12 students. For information please write, Secretary, S.P.A.D. 2 East 63 Street, New York 21, New York.

Common Cold Foundation

The Common Cold Foundation sponsored a conference on the common cold on February 13 in Chicago, Illinois. This is probably the first common cold conference ever held in which men of industry, science, and medicine discussed this complex disease. Dr. Thomas G. Ward, associate professor of bacteriology at Johns Hopkins University, was guest speaker at the conference, and his talk was followed by a panel discussion. More than 55 important industries in the Chicago area were represented by their medical directors, personnel, industrial and public relations officers, and industrial nurses.

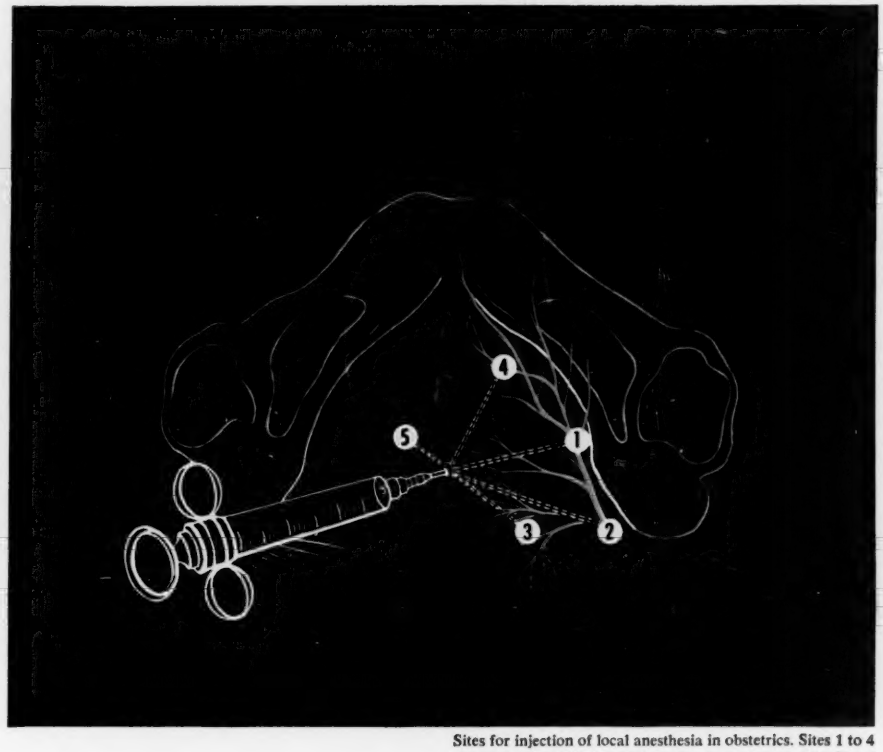
The Common Cold Foundation is a non-profit, free enterprise, and is incorporated under the laws of the State of Illinois. Its purpose is to create and disburse funds for use in developing research and investigation to the end that the common cold and its complications may be more adequately controlled, minimized, or eliminated from our national life.

World Medical Association

United States Committee, Inc.

The sixth general assembly of The World Medical Association will be held in Athens, Greece, October 12 to 16, 1952, and will be followed on October 17 by a meeting of the Medical Editors of the World.

About 25 members of the United States Committee attended the fifth general assembly in Stockholm, Sweden, last year. The World Medical Association hopes that many will be able to attend the sixth general assembly. While voting members of the assembly are restricted to two from each national member association, members of the United States Committee are entitled to attend as observers, and credential cards as such will be issued by this office if desired. Address World Medical Association, United States Committee, Inc., 2 East 103rd Street, New York 29, New York.



Sites for injection of local anesthesia in obstetrics. Sites 1 to 4 should be similarly injected on the contralateral side. Site 5 is for episiotomy. Adapted from Johnson, O. J.: Nerve Block in Painless Childbirth, J.A.M.A. *145*:401 (Feb. 10) 1951.

Pudendal Block in Obstetrics Simplified with **ALIDASE**

Using a local anesthetic with hyaluronidase, Heins[1] reports: "Complete perineal anesthesia is practically instantaneous. . . . The technique of pudendal block is greatly simplified. The operator does not have to inject the nerve per se, but infiltration in the vicinity of the nerve will accomplish an effective block."

Baum[2] states: "The use of hyaluronidase is found to be a safe and simple method for increasing the efficiency of pudendal block in obstetrics and for overcoming many of the objections to this type of obstetrical anesthetic."

ALIDASE® —highly purified, well tolerated brand of hyaluronidase—definitely shortens the period between completion of the block and establishment of operating analgesia. Swelling, induration and discomfort are almost negligible with Alidase.

[1]Heins, H. C.: Pudendal Block with Hyaluronidase, J. South Carolina M. A. *46*:309 (Oct.) 1950.
[2]Baum, F. E.: The Use of Hyaluronidase in Pudendal Block, Am. J. Obst. & Gynec. *60*:1356 (Dec.) 1950.

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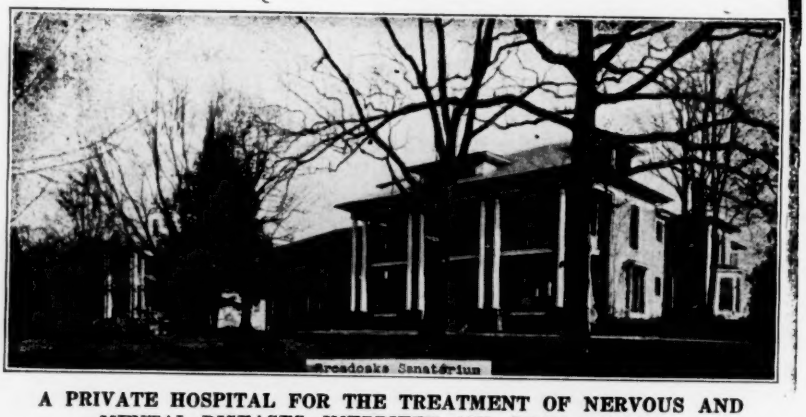

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Headache, a problem encountered in all kinds of medical practice, may occur in association with any of a variety of disorders, some organic, other purely functional.

Among the several types, functional headaches present the greatest problem because of their obscure etiology and recurrent nature.

Among these are:

Migraine (both classical and variant forms)
Tension headache
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Histaminic cephalgia

Wolff and his co-workers established that the pain of these headches is due to disturbance of the tonus of cranial blood vessels — hence the term *vascular headaches.*

The craniovascular changes associated with the several phases of the typical migraine attack are:

Vasoconstriction — to which the visual prodromata are attributable. It is possible to abort the attack during this phase in all but a few cases. (See treatment below.)

Vasodilatation — as the vessels lose their tone, exaggerated pulsations set in, resulting in the throbbing pain which characterizes vascular headache. Treatment for the attack is still effective during this phase. (See below.)

Vessel Edema — if the vasodilation continues for too long, vessel walls become edematous; this changes the character of the pain to a steady, intense aching. The attack can now no longer be checked, even with maximum dosage of specific drugs. *Moreover, sustained headache often induces reflex neck muscle tension, a source of residual pain.*

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GENERAL REFERENCES: DeJong, R.: Chicago M. Soc. Bull *54:* 106, 1951. Friedman, A.: Modern Headache Therapy, St. Louis, C. V. Mosby Co., 1951. Wolff, H.: Headache and Other Head Pain, N. Y., Oxford Univ. Press, 1948.

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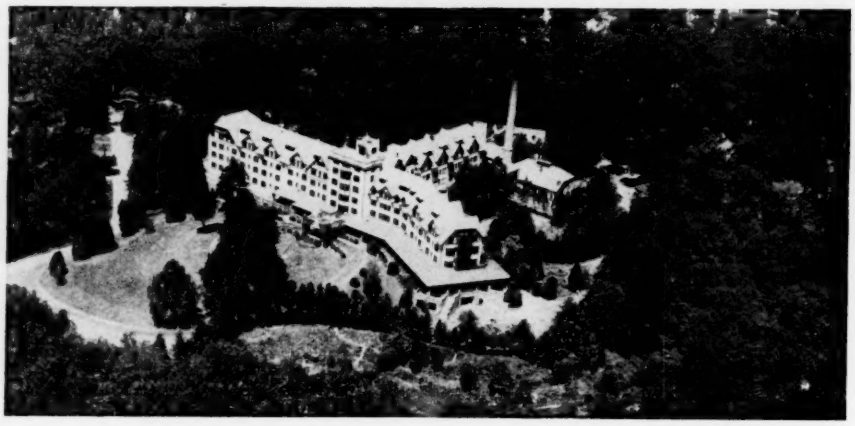

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1. Perloff, Wm. H., (1951) Treatment of the Menopause. II. Amer. J. Obst. & Gynec., 61:670, March. 2. Reich, W. J., et al. (1951), A Recent Advance in Estrogenic Therapy. I. American J. Obst. & Gynec., 62:427, August.

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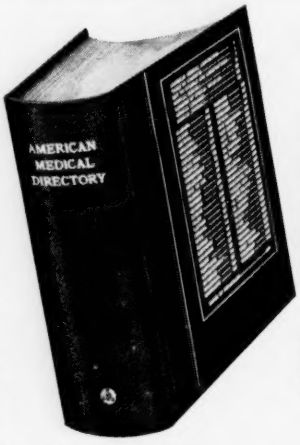

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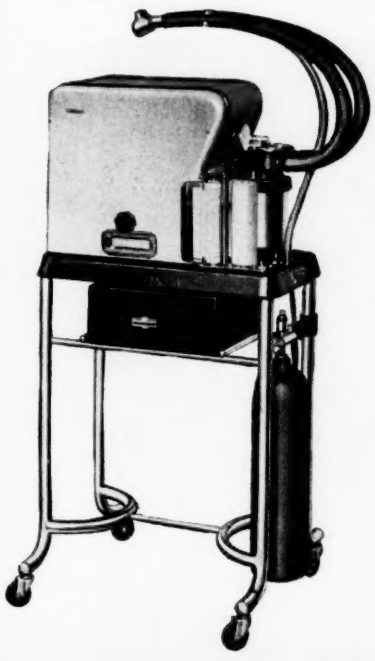

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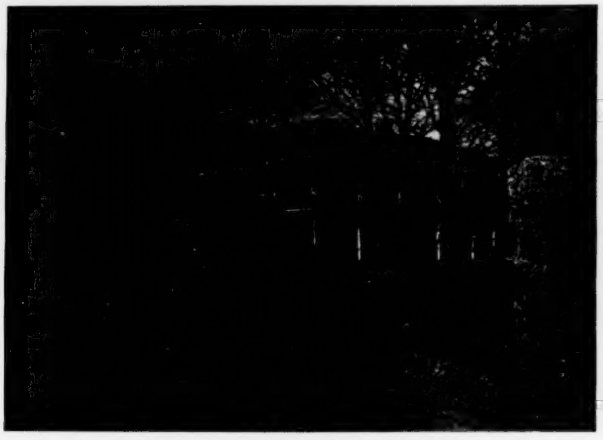

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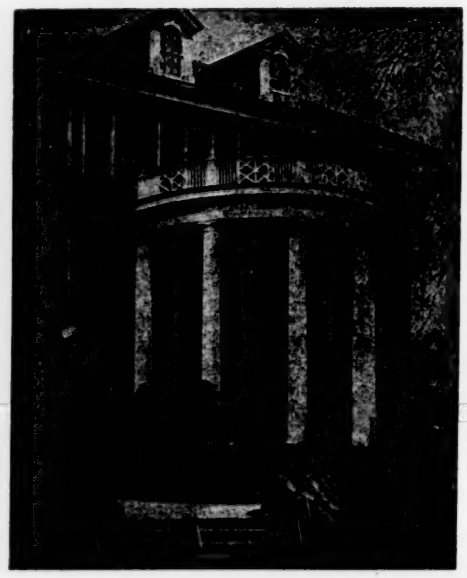

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